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Research Article

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Optimization of fermentation process for the production of functional carotenoids from *Rhodobacter sphaeroides* treated by nitrosoguanidine

Yi Tang[1], Xin Nie[2], Yao Yu[1], Xuemei Li[1], Li Zhang[1] and Zhiping Zhao[1*]

[1]*Department of Pharmaceutical Engineering, School of Chemical Engineering, Sichuan University of Science & Engineering, Zigong 643000, Sichuan, China*
[2] *College of Chemistry and Environmental Engineering, Sichuan University of Science & Engineering, Zigong 643000, Sichuan, China*

ABSTRACT

In the present study, single factors including inoculate amounts, fermentation temperature, fermentation duration, ratio of fermentation media volume to total flask volume were optimized for enhancing the production of carotenoids from nitrosoguanidine (NTG) treated Rhodobacter sphaeroides. The experimental results suggested that optimal single factor was: inoculate amounts 1%, fermentation temperature 37 ºC, fermentation duration 60 h, fermentation media volume to total flask volume 80%. Orthogonal experiments indicated that the optimal conditions for carotenoids production from NTG treated Rb. sphaeroides were: media volume to total flask volume 75%, fermentation time 60 h, fermentation temperature 37 ºC, inoculate amounts 4%. The present study will promote the large scale production of carotenoids from microorganisms.

Key words: carotenoids, *Rhodobacter sphaeroides*, optimization, fermentation

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INTRODUCTION

Carotenoids represent a group of valuable molecules for the pharmaceutical and food industries, which are the precursors for vitamin A and play very important roles in preventing human disease[1]. It has been reported that the adequate intake of carotenoids supplements may significantly reduce the risk of some chronic disease[2]. The scientific findings promote the rapid growth of carotenoids market. The carotenoids market in 2010 was about 1.2 billion USD and it will be 1.4 billion USD in 2018 with the considerable increase. Currently, carotenoids are commercially exploited as food colorant and feed additives and are being used in pharmaceutical, nutraceutical and cosmaceutical products[3]. However, the production of natural carotenoids is relatively low and can not be meet the increasing demands. In our previous study, we constructed a high-carotenoids *Rhodobacter sphaeroides* strain by nitrosoguanidine (NTG) mutagenesis. Metabolism pathways might be affected by NTG mutagenesis. Consequently, fermentation process for carotenoids production by the mutant *Rb. sphaeroides* strain is required. The present study focus on the optimization of fermentation process for the production of functional carotenoids from *Rhodobacter sphaeroides* treated by NTG and it will promote the large scale production of carotenoids used as feed additive from microorganisms.

EXPERIMENTAL SECTION

Extraction of total carotenoids from Rb. sphaeroides

Cell cultures of *Rb. sphaeroides* in fermentative medium were collected by centrifugation at 10,000 rpm for 10 min at 4 °C. The cell pellets were washed once with distilled water. The precipitate was subsequently resuspended in acetone and methanol mixture (acetone : methanol mixture = 7:2, v/v) at the ratio of 1:40. Then, the cells were broken by ultrasonic cooled with ice, 300 W, work for 3 s and stop for 5 s, continued for 15 min in the dark. The supernatant was collected by centrifugation at 10,000 rpm for 10 min. The supernatant was shaken at 150 rpm for 30

min and centrifuged at 12,000 rpm for 10 min in the dark to collect the supernatant containing carotenoids. Vacuum distillation and saponification reaction were used for further purification.

Determination of total carotenoids

The absorbance value of total carotenoids extracted from *Rb. sphaeroides* was evaluated by UV-vis spectrophotometer at 480 nm after suitable dilution. The total carotenoids yield (mg/L culture liquid) was calculated on the basis of culture broth volume according to the following formula[4].

$$\text{carotenoids yield (mg/L)} = \frac{ADV_1}{0.16V_2}$$

where *A* is the absorbance of diluted extract solution at 480 nm, D is the dilution ratio, V_1 is the volume of acetone and methanol mixture added, 0.16 is extinction coefficient of carotenoids, V_2 is the volume of fermentative liquid.

Effects of inoculate amounts on the yield of carotenoids

A single colony was inoculated in a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C for about 36 h. Precultures were inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 1%, 2%, 3%, 4% and 5% and grown in dark at 30 °C for 48 h. Total carotenoids were extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of oxygen tension on the yield of carotenoids

Oxygen tension was controlled by changing the ratio of liquid volume to total flask volume. A single colony was inoculated in a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C for about 36 h. Precultures were respectively inoculated into five 100-ml flasks containing 50, 60, 70, 80 and 90 ml of malate minimal media at 1% and grown in dark at 30 °C for 48 h.

Effects of fermentation time on the yield of carotenoids

A single colony was inoculated in a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C for about 36 h. Precultures were respectively inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 1% and grown in dark at 30 °C for 24, 36, 48, 60 and 72 h. Total carotenoids were extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Effects of fermentation temperature on the yield of carotenoids

A single colony was inoculated in a 50 ml-flask containing 40 ml of malate minimal media and grown under micro-aerobic conditions in dark at 30 °C for about 36 h. Precultures were inoculated into five 100-ml flasks containing 80 ml of malate minimal media at 1:100 and grown in dark at 23, 27, 30, 33 and 37 °C for 48 h. Total carotenoids were extracted from the cell cultures and quantified, respectively. The experiment was repeated three times.

Orthogonal experiments

Four factors and three levels obtained by single factor test were designed in the orthogonal experiment. Test factors and levels were listed in table 1. The experiment was repeated three times.

Table 1. Factors and levels for orthogonal experiment

Number	Media volume to total flask volume (%)	Time (h)	Temperature (°C)	Inoculate amounts (%)
1	75	36	30	1
2	75	48	33	2
3	75	60	37	4
4	80	36	30	1
5	80	48	33	2
6	80	60	37	4
7	85	36	30	1
8	85	48	33	2
9	85	60	37	4

RESULTS AND DISCUSSION

Effects of inoculate amounts on the production of carotenoids

Production of carotenoids from the mutant *Rb. sphaeroides* was influenced slightly by the inoculate amounts, as shown in Figure 1. Highest yield of carotenoids was produced when the inoculate amounts was 1%, followed by 4%

and 2%. Consequently, inoculate amounts of 1% was considered as the optimized inoculate amounts. For orthogonal experiment assay, inoculate amounts of 1%, 2% and 4% were measured.

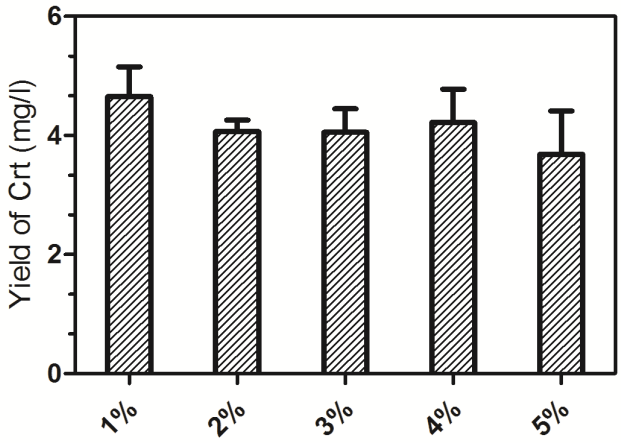

Figure 1. Effects of inoculate amounts on the production of carotenoids

Effects of oxygen tension on the production of carotenoids

Oxygen tension played critical roles in the production of carotenoids, as observed in Figure 2. When the liquid media covered 80% of the total volume of flask, highest yield of carotenoids was harvested. It has been well known that biosynthesis of carotenoids in *Rb. sphaeroides* is tightly regulated by oxygen tension[5]. Consequently, oxygen tension obtained by controlling the liquid media volume to the total flask volume of 80% was the optimized oxygen tension. The ratio of 75%, 80% and 85% were employed in orthogonal experiment.

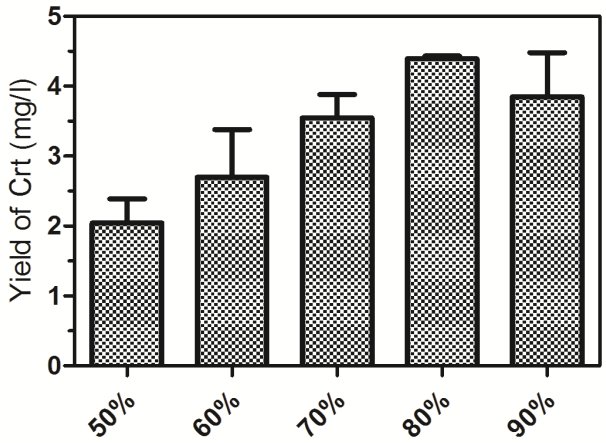

Figure 2. Effects of oxygen tension on the production of carotenoids

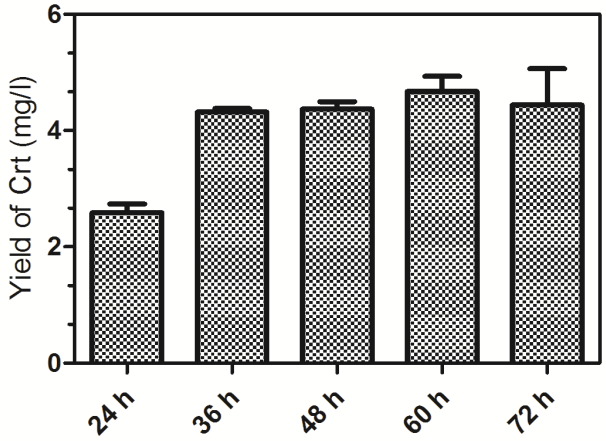

Figure 3. Effects of fermentation time on the production of carotenoids

Effects of fermentation time on the production of carotenoids

Production of carotenoids nearly was not influenced by fermentation time when fermentation time was more than 36 h, as seen in Figure 3. Highest yield of carotenoids was harvested when the fermentation time was 60 h. Consequently, fermentations for 36 h, 48 h and 60 were used in orthogonal experiment.

Effects of fermentation temperature on the production of carotenoids

Generally, fermentation temperature plays important roles in microorganism metabolisms. As can been seen in Figure 4, high productions of carotenoids were obtained when the fermentation temperature were 30, 33 and 37 ºC. So, 30, 33 and 37 ºC were used in orthogonal experiment.

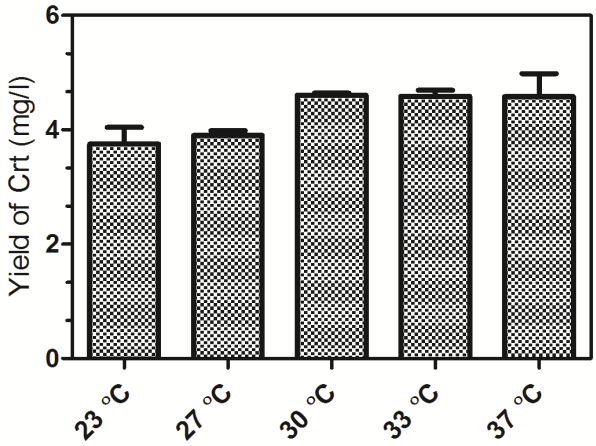

Figure 4. Effects of fermentation temperature on the production of carotenoids

Orthogonal experiment

To further optimize the fermentation process for higher production of carotenoids from mutant *Rb. sphaeroides*, orthogonal experiment was performed, as indicated in Figure 5. Based on the experimental results, it can be concluded that the best conditions for production of carotenoids from mutant *Rb. sphaeroides* were: media volume to total flask volume 75%, fermentation time 60 h, fermentation temperature 37 ºC, inoculate amounts 4%.

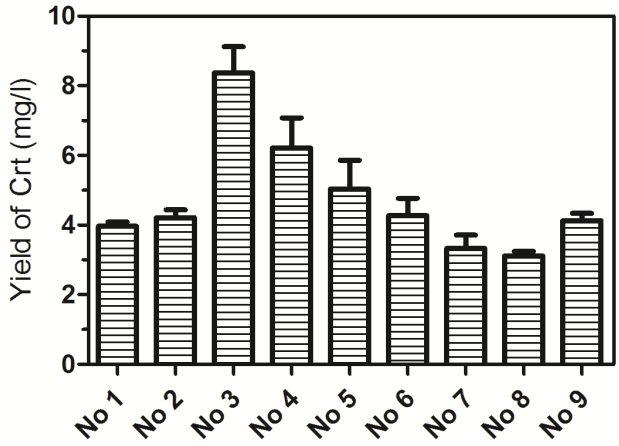

Figure 5. Orthogonal experiment results for the optimizing carotenoids production process from *Rb. sphaeroides*

CONCLUSION

(1) The single factor experiment results suggested that optimal single factor was: inoculate amounts 1%, fermentation temperature 37 ºC, fermentation duration 60 h, fermentation media volume to total flask volume 80%.
(2) The orthogonal experiments indicated that the optimal conditions for carotenoids production from NTG treated *Rb. sphaeroides* were: media volume to total flask volume 75%, fermentation time 60 h, fermentation temperature 37 ºC, inoculate amounts 4%.

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REFERENCES

[1] A Bonifacio; R Guidetti; T Altiero; V Sergo; L Rebecchi, *PLoS One*, **2012**, 7(1), e50162.
[2] J Fiedor; K Burda, *Nutrients*, **2014**, 6(2), 466-488.
[3]R Arimboor; RB Natarajan; KR Menon; LP Chandrasekhar; V Moorkoth, *Journal of Food Science and Technology-Mysore*, **2015**, 52(3), 1258-1271.
[4]DM Chen; YB Han; ZX Gu, *Process Biochemistry*, **2006**, 41(8), 1773-1778.
[5]HP Lang; CN Hunter, *Biochemical Journa*l, **1994**, 298(Pt1), 197-205.