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Optimization of β-carotene production by a newly isolated *Serratia marcescens* strain

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Abstract β-carotene is a commonly used food colorant. In this work, a novel β-carotene producing strain, *Serratia marcescens* RB3, was isolated and identified by physiological and biochemical tests, as well as 16S rDNA sequence analysis. The production of β-carotene by *S. marcescens* RB3 was identified through HPLC analysis. The cultivation conditions for β-carotene production by *S. marcescens* RB3 were optimized as 2.0% lactose, 2.0% peptone, 0.3% beef extract, 1.0% NaCl supplemented with 0.05% Fe^{2+}, pH 6.0 and 30ºC. Under the optimal conditions, the yield of β-carotene achieved 2.45 μg/mL.

Keywords: β-carotene, optimization, pigment, *Serratia marcescens.*

INTRODUCTION

The application of synthetic colorants in food industry has resulted in safety concerns due to their potentially harmful effects on human health (Velmurugan et al. 2010). Pigments derived from natural sources have emerged as an important alternative to synthetic food colorants. Natural pigments with an annual growth rate of 5-10%, have now comprised 31% of the worldwide colorant market, compared to 40% for synthetic colorants (Downham and Collins, 2000; Mapari et al. 2010).

Natural β-carotene is an orange-yellow pigment of carotenoid family that is widely used as a food colorant. The global market of β-carotene is estimated to surpass USD $280 million in 2015 (Ribeiro et al. 2011). β-carotene is very attractive as natural food colorant due to its antioxidant and pro-vitamin activities which provide additional value to the products (Paz et al. 2012). Furthermore, β-carotene was proved to have beneficial influence on iron and zinc bioaccessibilities (Gautam et al. 2010). The main sources of natural β-carotene include extraction from vegetable resources and microbial fermentation (Ribeiro et al. 2011).

The production of natural colorants through fermentation has a number of advantages, such as cheaper production, higher yields, possibly easier extraction, less batch-to-batch variations, no lack of raw materials, and no seasonal variations (Mortensen, 2006; Mapari et al. 2010). β-carotene can be produced by numerous microorganisms such as *Blakeslea trispora* (fungi), *Rhodotorula* spp., and *Saccharomyces cerevisiae* (yeast), and *Dunaliella bardawil* (microalgae) (Malisorn and Suntornsuk, 2009; Mogedas et al. 2009; Nanou and Roukas, 2011). Although there are also some bacteria species which produce β-carotene as main carotenoid, these species must have the central metabolism inhibited by inorganic salts and urea or must be genetically engineered (Ribeiro et al. 2011). Little information is known about other β-carotene producing bacterial species. In this work, we reported the

identification of a new *Serratia marcescens* strain which produce β-carotene as the main pigment. The production of β-carotene by this strain was also optimized.

MATERIALS AND METHODS

Microorganism and medium

Strain RB3 was isolated from red-pigmented colonies on decayed woods and maintained on nutrient agar slants at 4ºC.

Strain identification

The biochemical tests were performed according to the methods described by Dong and Cai (2001). 16S rDNA sequencing was done by Guangdong Detection Center of Microbiology (Guangdong, China). The 16S rDNA sequences of the isolated strain were aligned with other sequences from the GenBank database by using BLAST programme. Phylogenetic tree was constructed by MEGA 4.0 using the neighbour-joining method.

Preparation of pigment extraction

Strain RB3 was activated on nutrient agar slants at 30ºC for 48 hrs, and was then inoculated into Erlenmeyer flasks (250 mL) containing 40 mL of nutrient broth. The cultures were incubated at 30ºC on a rotary shaker at 200 rpm for 48 hrs. Liquid culture was centrifuged at 4000 rpm for 15 min. For pigment extraction, the washed cell pellets was vortexed for 60 min in the same volume of 3 mol/L HCl. The mixture was then incubated in boiling water for 4 min and was then chilled quickly. After 10 min centrifugation at 4000 rpm, the pellets were washed twice with distilled water and vortexed for 30 min in the same volume of acetone. The pigment supernatant was obtained by centrifugation at 4000 rpm for 20 min and its pigment content was determined according to the absorbance at 475 nm using a spectrophotometer (TU-1901, Purkinje General, China).

HPLC analysis of β-carotene

β-carotene standard sample (Sigma, St. Louis, MO) was dissolved in methanol with a concentration of 2-26 µg/mL. A standard calibration curve was done for quantification. For preparation of sample solution, 20 mL of liquid culture was centrifuged at 5000 rpm for 15 min, and the pellet was washed to colourless with water. The obtained cell pellets were extracted twice with a mixture of petroleum ether-acetone (50:50, v/v) and were freeze-dried. The resulting sample was dissolved in 2 mL of methanol, filtrated through a 0.45 µm membrane and analyzed by HPLC (Waters 2695, Milford, MA, USA). The HPLC analysis was performed on a symmetry C18 column (150 x 4.6 mm, 5 µm particle size) (Waters, Milford, MA). The UV detector was operated at 450 nm and the column temperature was maintained at 25ºC. The mobile phase was acetonitrile-tetrahydrofuran mixture solvent (60:40, v/v) with a flowrate of 1 mL/min.

Optimization of β-carotene production

Several factors including carbon source, metal ions, initial pH value and temperature were optimized for β-carotene production based on beef extract-peptone liquid medium. According to the results of single-factor experiment, the orthogonal experiment was designed as four factors (lactose, peptone, beef extract and NaCl) and three levels (Table 1).

Table 1. Orthogonal experiment for β-carotene production.

Levels	Factors			
	Lactose	Peptone	Beef extract	NaCl
1	1%	1.0%	0.3%	0.5%
2	2%	1.5%	0.5%	1.0%
3	3%	2.0%	0.7%	2.0%

RESULTS AND DISCUSSION

Strain identification

The isolated strain RB3 showed a strong ability to produce red pigment (Figure 1). The strain RB3 was gram negative and motile short rod. The colonies were smooth, red and opaque, with regular edge. The physiological and biochemical characteristics of strain RB3 are summarized in Table 2. The results suggested that the strain RB3 is a *Serratia* species. The BLAST results of 16S rDNA sequence indicated that the strain RB3 showed the highest similarity (> 99%) with *Serratia marcescens*. A phylogenetic tree was constructed based on the partial 16S rDNA sequences of strain RB3 (Figure 2). The results revealed that RB3 strain was closely related to *S. marcescens*. Thus, based on morphological characterization, biochemical tests and sequence analysis, the isolated strain RB3 was finally identified as *S. marcescens*.

Table 2. Physiological and biochemical characteristics of the isolated strain RB3.

Characteristics	Strain RB3	Characteristics	Strain RB3
Anaerobic	+	Fermentation of:	
H_2S	-	Maltose	+
Gelatin hydrolysis	+	Rhamnose	-
Starch hydrolysis	-	Mannose	+
Indole test	-	Glucose	+
Voges proskauer test	+	Arabinose	-
Nitrate reduction	+	Lactose	+
Methyl red test	+	Mannitol	+
Catalase	+	Inositol	-
Oxidase	-	Sorbitol	+
Phenylalanine deaminase	-	Sucrose	+
Lysine decarboxylase	+	Fucose	+

Identification of β-carotene produced by strain RB3

According to the HPLC analysis (Figure 3a), the retention time of β-carotene standard was 3.17 min. With regard to the carotenoids extracted from *S. marcescens* RB3, a peak with the same retention time appeared (Figure 3b). Thus, the extracted pigment was identified as β-carotene. *S. marcescens* is characterized by its production of the red pigment prodigiosin, which is well-known for its antimicrobial and anticancer activities (Kalivoda et al. 2010). However, the production of β-carotene by *S. marcescens* has not been reported. Since *S. marcescens* is a ubiquitous Gram-negative bacterium found in soil, water, plant surface and insects, it might be used as a new source for β-carotene production.

Optimization of β-carotene production

Choudhari and Singhal (2008) found that *Blakeslea trispora* could not produce β-carotene without carbon source. The influence of different carbon sources on the pigment production, including glucose, sucrose, lactose, maltose and starch, was investigated with a final concentration of 2%. Among the several compounds tested, lactose was proved to be the most suitable carbon source, with 1.209 µg/mL of pigment obtained (Figure 4a). It may be due to that lactose can be easily assimilated in the metabolic pathway for biosynthesis of β-carotene. For the fungus *Blakeslea trispora*, glucose was reported to give the maximum yield of β-carotene (Choudhari and Singhal, 2008).

The effect of metal ions on β-carotene concentration was shown in Figure 4b. Only Fe^{2+} and Fe^{3+} improved the pigment production, while other metal ions inhibited the production of β-carotene. The positive influence of Fe^{3+} on carotene production was found by Filotheou et al. (2012). It might be attributed to the high amount of hydroxyl radical generated by the Fenton reaction, since the stimulation of biosynthesis of carotenes by oxidative stress has been observed in *Blakeslea trispora* (Nanou and Roukas, 2011).

The pH of the culture medium is an important factor for metabolite production in cells. Figure 4c depicted the effect of pH on pigment biosynthesis by *S. marcescens* RB3. β-carotene content was significantly affected by pH, and the maximum β-carotene production was obtained at pH 6.0 (Figure 4c). Similarly, the production of β-Carotene by yeast *Rhodotorula acheniorum* was favoured at a lower pH (about 5.5) (Nasrabadi and Razavi, 2011). Temperature is another critical parameter that has to be controlled in fermentation. Maximal β-carotene production was achieved at 30ºC for strain RB3, and higher temperature decreased the β-carotene production (Figure 4d). Similar result was also reported for *Rhodotorula glutinis* (Malisorn and Suntornsuk, 2008). However, better titer of β-carotene by metabolically engineered *Escherichia coli* cells was obtained at 37ºC rather than 28ºC, which might be attribute to the increased level of IPP through more activated foreign MVA pathway as well as higher glycolysis pathway flux (Kim et al. 2009).

The orthogonal experiment indicated that the optimal culture conditions were: 2.0% lactose, 2.0% peptone, 0.3% beef extract, 1.0% NaCl supplemented with 0.05% Fe^{2+}, pH 6.0, and with fermentation temperature of 30ºC (Table 3). The impact of different factor was in decreasing order of: peptone>NaCl>lactose>beef extract. Under the optimal condition, the pigment yield of RB3 strain was 2.45 μg/mL. The medium optimization resulted in a 69.2% increase of pigment production.

Table 3. Orthogonal experiment of the β-carotene production by strain RB3.

No.	Lactose	Peptone	Beef extract	NaCl	β-carotene production (μg/ml)	β-carotene production (μg/ml)
1	1	1	1	1	2.105	2.043
2	1	2	2	2	1.884	1.866
3	1	3	3	3	1.593	1.548
4	2	1	2	3	1.302	1.269
5	2	2	3	1	1.832	1.650
6	2	3	1	2	2.390	2.454
7	3	1	3	2	1.680	1.779
8	3	2	1	3	1.285	1.276
9	3	3	2	1	2.008	2.094
k_1	1.84	1.69	1.93	1.96		
k_2	1.82	1.63	1.74	2.01		
k_3	1.69	2.01	1.68	1.38		
R	0.15	0.38	0.25	0.63		

CONCLUDING REMARKS

Serratia marcescens RB3 has been shown a potential strain for β-carotene production. Its production was affected by temperature, pH and medium composition. By using statistical experimental methods, the fermentation conditions were optimized as follows, fermentation temperature 30ºC, initial pH 6.0, and the cultural medium was composed by 2.0% lactose, 2.0% peptone, 0.3% beef extract, 1.0% NaCl and 0.05% Fe^{2+}. The maximal pigment yield was 2.45 μg/mL. These results suggest that strain RB3 is worthy of further study for β-carotene industrialization.

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REFERENCES

CHOUDHARI, S. and SINGHAL, R. (2008). Media optimization for the production of β-carotene by *Blakeslea trispora*: A statistical approach. *Bioresource Technology*, vol. 99, no. 4, p. 722-730. **[CrossRef]**

DONG, X.Z. and CAI, M.Y. (2001). *Manual of systematic identification of common bacteria*. Science Press, 419 p. ISBN 978-7-030-08460-6.

DOWNHAM, A. and COLLINS, P. (2000). Colouring our foods in the last and next millennium. *International Journal of Food Science & Technology*, vol. 35, no. 1, p. 5-22. **[CrossRef]**

FILOTHEOU, A.; NANOU, K.; PAPAIOANNOU, E.; ROUKAS, T.; KOTZEKIDOU, P. and LIAKOPOULOU-KYRIAKIDES, M. (2012). Application of response surface methodology to improve carotene production from synthetic medium by *Blakeslea trispora* in submerged fermentation. *Food and Bioprocess Technology*, vol. 5, no. 4, p. 1189-1196. **[CrossRef]**

GAUTAM, S.; PLATEL, K. and SRINIVASAN, K. (2010). Influence of β-carotene-rich vegetables on the bioaccessibility of zinc and iron from food grains. *Food Chemistry*, vol. 122, no. 3, p. 668-672. **[CrossRef]**

KALIVODA, E.J.; STELLA, N.A.; ASTON, M.A.; FENDER, J.E.; THOMPSON, P.P.; KOWALSKI, R.P. and SHANKS, R.M.Q. (2010). Cyclic AMP negatively regulates prodigiosin production by *Serratia marcescens*. *Research in Microbiology*, vol. 161, no. 2, p. 158-167. **[CrossRef]**

KIM, J.H.; KIM, S.W.; NGUYEN, D.Q.A.; LI, H.; KIM, S.B.; SEO, Y.G.; YANG, J.K.; CHUNG, I.Y.; KIM, D.H. and KIM, C.J. (2009). Production of β-carotene by recombinant *Escherichia coli* with engineered whole mevalonate pathway in batch and fed-batch cultures. *Biotechnology and Bioprocess Engineering*, vol. 14, no. 5, p. 559-564. **[CrossRef]**

MALISORN, C. and SUNTORNSUK, W. (2008). Optimization of β-carotene production by *Rhodotorula glutinis* DM28 in fermented radish brine. *Bioresource Technology*, vol. 99, no. 7, p. 2281-2287. **[CrossRef]**

MALISORN, C. and SUNTORNSUK, W. (2009). Improved β-carotene production of *Rhodotorula glutinis* in fermented radish brine by continuous cultivation. *Biochemical Engineering Journal*, vol. 43, no. 1, p. 27-32. **[CrossRef]**

MAPARI, S.A.S.; THRANE, U. and MEYER, A.S. (2010). Fungal polyketide azaphilone pigments as future natural food colorants? *Trends in Biotechnology*, vol. 28, no. 6, p. 300-307. **[CrossRef]**

MOGEDAS, B.; CASAL, C.; FORJÁN, E. and VÍLCHEZ, C. (2009). β-Carotene production enhancement by UV-A radiation in *Dunaliella bardawil* cultivated in laboratory reactors. *Journal of Bioscience and Bioengineering*, vol. 108, no. 1, p. 47-51. **[CrossRef]**

MORTENSEN, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, vol. 78, no. 8, p. 1477-1491. **[CrossRef]**

NANOU, K. and ROUKAS, T. (2011). Stimulation of the biosynthesis of carotenes by oxidative stress in *Blakeslea trispora* induced by elevated dissolved oxygen levels in the culture medium. *Bioresource Technology*, vol. 102, no. 17, p. 8159-8164. **[CrossRef]**

NASRABADI, M.R.N. and RAZAVI, S.H. (2011). Optimization of β-carotene production by a mutant of the lactose-positive yeast *Rhodotorula acheniorum* from whey ultrafiltrate. *Food Science and Biotechnology*, vol. 20, no. 2, p. 445-454. **[CrossRef]**

PAZ, E.D.; MARTÍN, Á. ESTRELLA, A.; RODRÍGUEZ-ROJO, S.; MATIAS, A.A.; DUARTE, C.M.M. and COCERO, M.J. (2012). Formulation of β-carotene by precipitation from pressurized ethyl acetate-on-water emulsions for application as natural colorant. *Food Hydrocolloids*, vol. 26, no. 1, p. 17-27. **[CrossRef]**

RIBEIRO, B.D.; BARRETO, D.W. and COELHO, M.A.Z. (2011). Technological aspects of β-carotene production. *Food and Bioprocess Technology*, vol. 4, no. 5, p. 693-701. **[CrossRef]**

VELMURUGAN, P.; KAMALA-KANNAN, S.; BALACHANDAR, V.; LAKSHMANAPERUMALSAMY, P.; CHAE, J.C. and OH, B.T. (2010). Natural pigment extraction from five filamentous fungi for industrial applications and dyeing of leather. *Carbohydrate Polymers*, vol. 79, no. 2, p. 262-268. **[CrossRef]**

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Figures

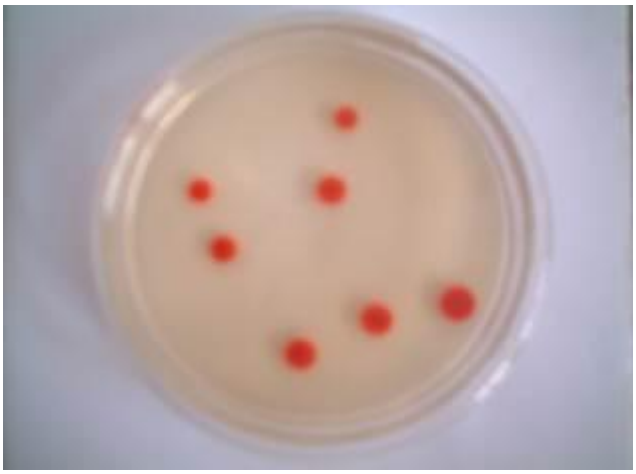

Fig. 1 Red-pigment producing strain RB3 isolated from decayed woods.

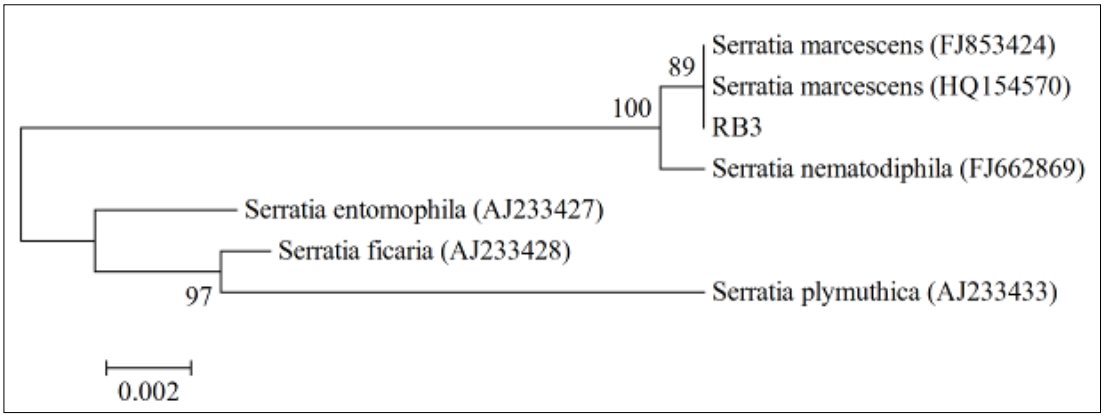

Fig. 2 Phylogenetic tree based on the 16S rDNA sequence of strain RB3.

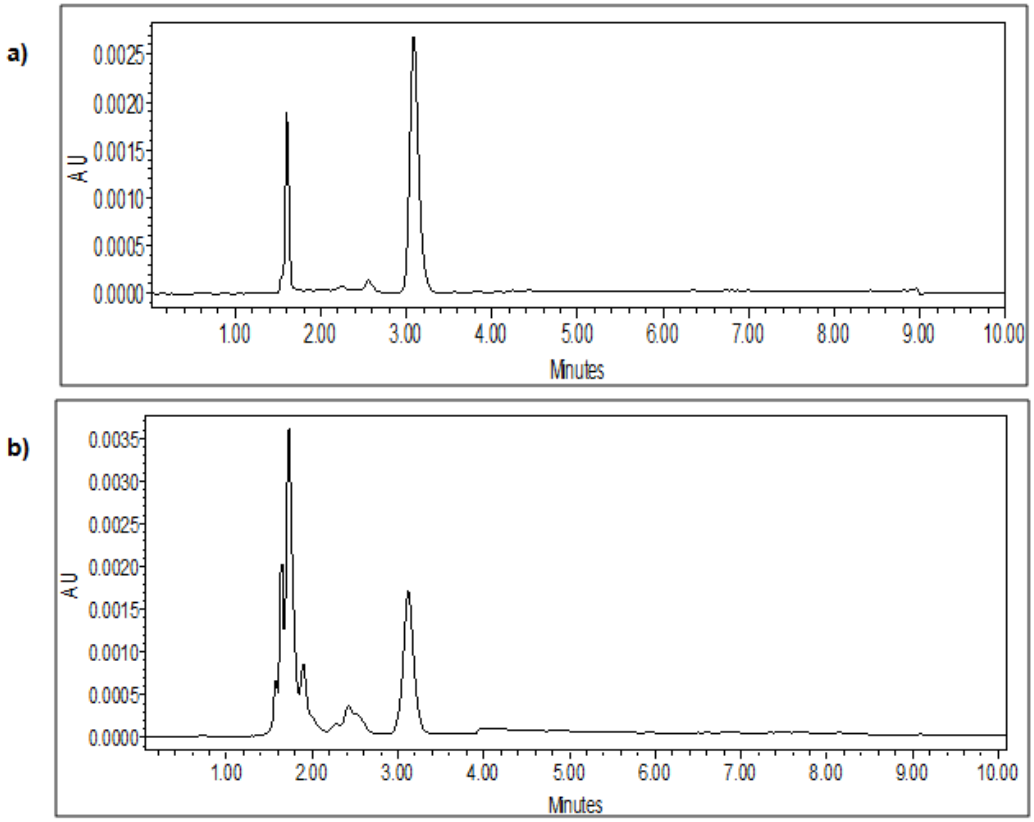

Fig. 3 HPLC analysis of β-carotene standard (a) and fermentation sample (b).

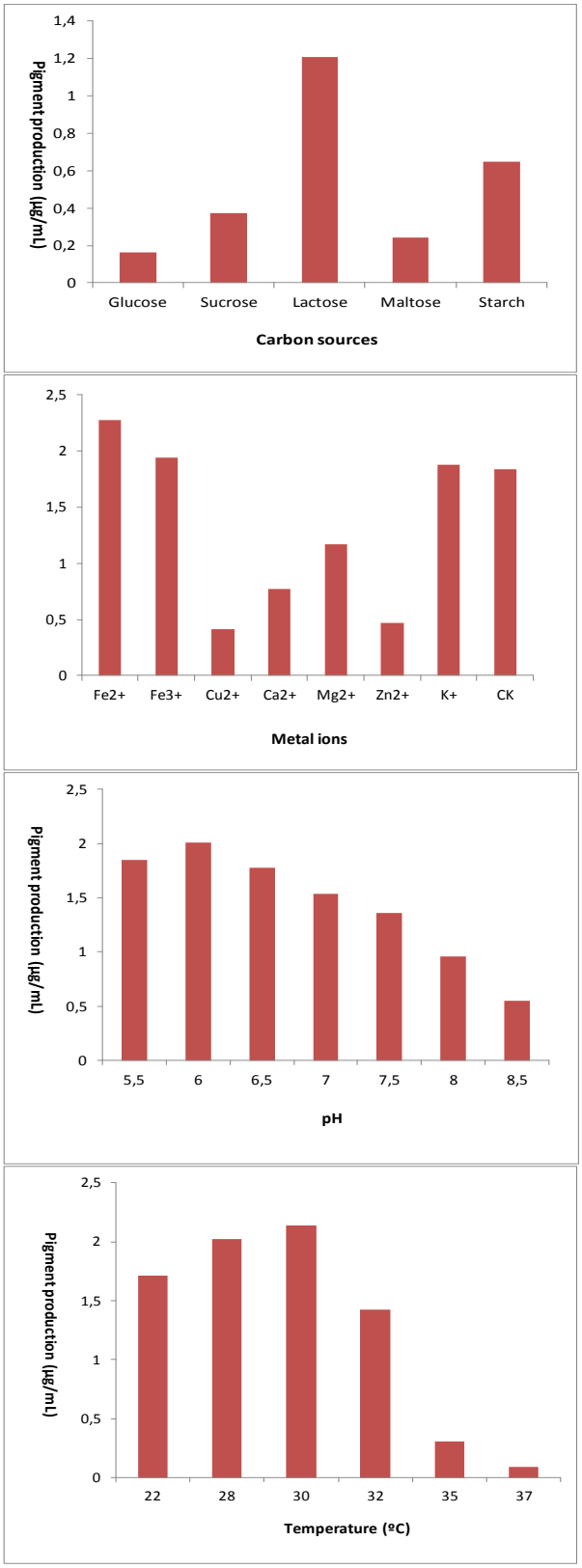

Fig. 4 Effects of various parameters on β-carotene production by strain RB3. (a) carbon sources; (b) metal ions; (c) pH; (d) temperature.