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Research article

Carotenoid production at various salinities in bacterium *Rhodopseudomonas palustris*

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Abstract

Rhodopseudomonas palustris, a phototrophic purple non-sulfur bacterium, is well known as a Gram negative, rod-shaped and motile cell. The effect of salinity on total carotenoid production and composition produced in *R. palustris* isolated from a paddy field was investigated. *R. palustris* was cultured for 30 d in *Rhodopseudomonas* medium, which was adjusted to four levels of salinity (0 parts per thousand, ppt; 10 ppt, 20 ppt and 30 ppt). The results revealed that mean (± SD) total carotenoid accumulation of *R. palustris* cultured in 0 ppt medium was faster at 9 d culture (46.30 ± 18.10 mg/L) than in 10 ppt medium at 21 d culture (57.27 ± 9.43 mg/L). At 30 d culture, the mean total carotenoid content at 0 ppt salinity (232.30 ± 52.53 mg/L) was similar with those produced at 10 ppt salinity (154.81 ± 41.47 mg/L, $p > 0.05$). The culture at 20 and 30 ppt salinity had minimal carotenoid content during the 30 d of the experiment. There was a fluctuating pattern of cell growth based on the dry cell weight for all salinity levels. Carotenoids and bacteriochlorophyll a were found in the extractions of *R. palustris* cultured in 0 ppt and 10 ppt salinity using spectrophotometry and thin-layer chromatography techniques. Interestingly, the results confirmed the synthesis of astaxanthin which was not commonly produced by this bacterium.

Introduction

Carotenoids mainly present as a yellow to red color that absorbs blue light (440–490 nm). Their chemical structures consist of eight units of isoprene (C_5) linked head to tail as a so-called tetraterpene (C_{40}). Carotenoids can be synthesized by plants and microorganisms. They have been an important dietary supplement in aquaculture industry for a long time because commercial aquatic animals cannot synthesize them by themselves. In aquaculture, the outstanding types of carotenoids that have been the focus are β-carotene, zeaxanthin, canthaxanthin and astaxanthin (Sanchez et al., 2013) because they enhance meat colors (Chandi and Gill, 2011) and help the boost immune system (Niu et al., 2014; Chien and Shiau, 2005).

Because of their important roles and applications, there is a projected 3.5% increase in demand for carotenoids globally between 2016 and 2021 (Globenewswire, 2013). Due to the growth of carotenoid consumption, there have been many attempts to find new sources for carotenoid production from natural microorganisms

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such as the green microalga *Dunaliella salina*, the fungus *Blakeslea trispora* and the yeast *Rhodotorula* spp. which are well known as major β-carotene accumulating organisms (Choudhari and Singhal, 2008; Malisorn and Suntornsuk, 2008; Morowvat and Ghasemi, 2016; Alipour et al., 2017). Furthermore, the famous astaxanthin-producing microorganisms are the green micro alga *Haematococcus pluvialis* and the yeast *Xanthophyllomyces dendrorhous* as well as the marine bacteria *Agrobacterium aurantiacum* and *Scenedesmus obliquus* (Yokoyama and Miki, 1995; Kobayashi et al., 2001; Hu et al., 2006; Qin et al., 2008).

Chemical substances and physical conditions can be used effectively to stimulate carotenoid production from microorganisms. Nutrients (sucrose or ammonium sulfate) added into medium of the yeast *Rhodotorula mucilaginosa* increased total carotenoids (Aksu and Eren, 2005; Aksu and Eren, 2007), while $MgSO_4$, Na_2HPO_4, $FeSO_4$ and Na_2CO_3 can induce carotenoid synthesis and significantly boost the yield of the bacterium *Rhodobacter sphaeroides* (Chen et al., 2006). In addition, pH also affects carotenoid production (Aksu and Eren, 2005, 2007). Furthermore, new sources of natural microorganisms, which induce carotenoid production by using several stimulants, can eliminate the problem of chemical residues, develop food security and reduce land competition and the cost of production.

The bacterium *Rhodopseudomonas palustris* is a phototrophic, purple, non-sulfur bacterium and is characterized as rod-shaped, Gram negative and has motile cells (Imhoff, 2005). Its cells generally consist of photosynthetic pigments such as bacteriochlorophyll and carotenoids (Imhoff et al., 2005). *R. palustris* normally accumulates carotenoid series such as lycopene, rhodopin, rhodovibrin and spirilloxanthin (Brown, 1968; Mehrabi et al., 2001). Some researchers have shown that *R. palustris* can synthesize carotenoid production by using stimulants such as light sources or salinity (Kuo et al., 2012; Thanomchaisanit, 2013).

Previous research has revealed that the mechanism of high salinity stress could induce pigment production in microorganisms such as microalgae (Gómez et al., 2003; Paliwal et al., 2015). There have been few reports of such evidence for photosynthetic bacteria such as *Rhodopseudomonas* sp. (Wang et al., 2017). In particular, carotenoid synthesis under salinity stress conditions in *R. palustris* has not been mentioned. Therefore, the focus of this research was to induce *R. palustris* for carotenoid production and to determine the productivity at various salinity levels. The results of the study will provide more knowledge about the new resource of carotenoid-producing microorganisms.

Materials and Methods

Sample and media preparation

R. palustris isolated from water in paddy field was kindly donated by Dr Sirapan Sukondhasingha, Faculty of Veterinary Technology, Kasetsart University, Bangkok, Thailand. Various salinity levels (0 parts per thousand, ppt; 10 ppt, 20 ppt and 30 ppt) were prepared by mixing seawater with distilled water. The cultures were triplicated and carried out in *Rhodopseudomonas* medium (ATCC Medium 543) at pH 7.0 ± 0.2, containing: 2.5 g/L sodium succinate, 1.25 g/L $(NH_4)_2SO_4$, 0.9 g/L K_2HPO_4, 0.6 g/L KH_2PO_4, 0.5 g/L yeast extract, 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.07 g/L $CaCl_2$, 0.003 g/L ferric citrate and 0.002 g/L EDTA, prior to sterilization at 121°C for 15 min (Atlas, 2010).

Extraction and analysis of total carotenoids

Total carotenoid extraction and quantitative analysis were carried out according to Homthong (1998). Culture samples of 5 mL were centrifuged at 9,700×*g* and 4°C for 10 min and then the medium was washed out using distilled water. Cell pellets were extracted using a mixture of acetone:methanol (40:60) and sonicated until colorless. After filtering, the absorbance of the supernatant was measured at 480 nm using a spectrophotometer and then evaporated and flushed with nitrogen gas. Total carotenoids were calculated using the 1% extinction coefficient of 2,500.

Thin-layer chromatography separation and analysis of carotenoids

Thin-layer chromatography (TLC) was prepared using the method of Quach et al. (2004). TLC plates (10 cm × 10 cm) were cut from commercially available sheets. The concentrated carotenoid samples dissolved in acetone:methanol (40:60) solution were spotted onto silica gel TLC sheets and developed using the mobile phase of acetone:hexane (30:70) at room temperature. Astaxanthin was identified using shrimp extract and an authentic standard (Funakoshi, Japan).

Growth determination

Bacterial cell growth was determined based on the dry cell weight. A collection of 1.5 mL from each sample was centrifuged at 9,700×*g* and 4°C for 10 min. The samples were washed twice with distilled water and then dried overnight at 105°C until they had constant weight, before being cooled in a desiccator and reweighed.

Statistical analysis

The results were presented as mean ± SD. All data were subjected to one-way analysis of variance. Means were compared using Tukey's honestly significant difference test with a critical value at $p < 0.05$.

Results and Discussion

The effects of salinity levels at 0 ppt, 10 ppt, 20 ppt and 30 ppt on total carotenoid production and growth of *R. palustris* have been investigated for 30 d with the observations recorded every 3 d. The results showed that all cell suspensions were transparent yellow at the starting time and changed to be turbid within 24 hr. The cell suspension with 0 ppt salinity turned to pale red after the first 6 d of culture, while the cell suspension with 10 ppt salinity was a clear pale red after 12 d of culture. However, the cell suspensions of 20 and 30 ppt salinity were still cloudy yellow at 30 d of culture.

Total carotenoid production

The mean ± SD total carotenoid contents in the bacterial cultures are shown in Table 1 and ranged from 0.48 ± 0.26 mg/L to 242.79 ± 38.25 mg/L across the range of salinity (0 ppt, 10 ppt, 20 ppt and 30 ppt). The mean total carotenoid contents at 0 ppt were significantly lower at 3 d and 6 d of culture (5.60 ± 1.39 mg/L and 8.69 ± 2.83 mg/L, respectively) than those at the same salinity level on other successive days. The mean contents rapidly increased and were significantly different from 9 d of culture (46.30 ± 18.10 mg/L) to 27 d of culture (242.79 ± 38.25 mg/L). Moreover, the mean total carotenoid contents at 10 ppt were significantly lower between 3 d of culture (1.76 ± 0.38 mg/L) and 18 d of culture (9.60 ± 7.62 mg/L). These contents greatly increased from 21 d of culture (57.27 ± 9.43 mg/L) to 30 d of culture (154.81 ± 41.47 mg/L).

At the 20 ppt salinity level, the mean total carotenoid content was significantly higher at 30 d of culture (11.12 ± 2.33 mg/L) than during first 27 d of culture. Similarly, the mean carotenoid content at 30 ppt salinity was highest at 30 d of culture (4.72 ± 0.38 mg/L) and was significantly different from that during at 27 d of culture.

Comparison of the mean total carotenoids among the four salinity levels in each duration (Fig. 1) indicated a significant content at 0 ppt salinity during the first six days, although all salinity levels had low contents. From 9–18 d of culture, the mean contents at 0 ppt salinity sharply increased and were significantly higher than the others. However, Sarada et al. (2002) showed that *H. pluvialis* cultured in medium without NaCl and sodium acetate addition had the lowest carotenoid yields. At 21 d of culture, the carotenoid content at 10 ppt salinity suddenly rose but was significantly lower than at 0 ppt salinity. At the end of the 30 d of culture, the mean carotenoid content at 0 ppt salinity was similar to those produced at 10 ppt salinity. In contrast, Thanomchaisanit (2013) reported that carotenoid accumulation in *Rhodopseudomonas* sp. was maximized in 10 ppt culture.

Table 1 Total carotenoids accumulation of *R. palustris* cultured at different salinity levels in *Rhodopseudomonas* medium during 30 d

Culture period (d)	Total carotenoids (mg/L)			
	Salinity level (ppt)			
	0	10	20	30
3	5.60 ± 1.39^{a}	1.76 ± 0.38^{a}	2.14 ± 0.14^{ab}	1.64 ± 0.62^{ab}
6	8.69 ± 2.83^{a}	1.08 ± 0.77^{a}	0.60 ± 0.07^{a}	0.88 ± 0.20^{a}
9	46.30 ± 18.10^{b}	5.49 ± 1.37^{a}	1.40 ± 0.60^{ab}	1.36 ± 0.39^{ab}
12	86.33 ± 22.06^{bc}	8.36 ± 4.36^{a}	1.48 ± 0.33^{ab}	1.94 ± 0.71^{ab}
15	130.39 ± 30.24^{c}	9.24 ± 1.90^{a}	2.25 ± 1.20^{ab}	1.29 ± 0.50^{ab}
18	161.42 ± 23.01^{c}	9.60 ± 7.62^{a}	1.77 ± 0.64^{ab}	1.60 ± 1.46^{ab}
21	170.37 ± 23.05^{c}	57.27 ± 9.43^{b}	1.57 ± 1.39^{ab}	0.48 ± 0.26^{a}
24	203.46 ± 45.47^{cd}	117.07 ± 38.48^{c}	3.65 ± 0.94^{b}	3.11 ± 0.91^{bc}
27	242.79 ± 38.25^{d}	140.58 ± 30.81^{c}	2.04 ± 0.55^{ab}	1.55 ± 0.44^{ab}
30	232.30 ± 52.53^{d}	154.81 ± 41.47^{c}	11.12 ± 2.33^{c}	4.72 ± 0.38^{c}

mean ± SD values with different lowercase superscript letters within each column denote significant ($p < 0.05$) differences between groups.

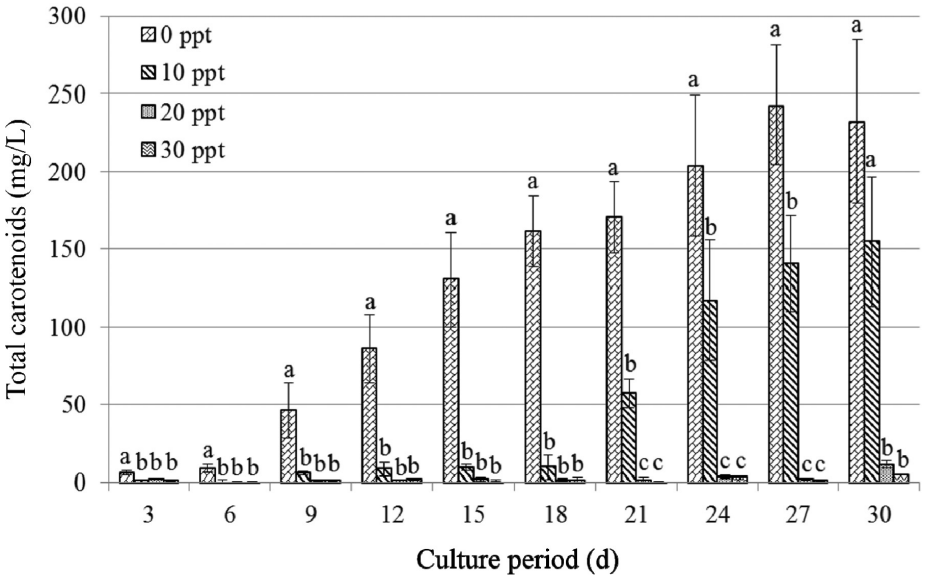

Fig. 1 Total carotenoid accumulation of *R. palustris* cultured at salinity levels of 0–30 parts per thousand (ppt) every 3 d for 30 d. Values are means of triplicates, error bars show ± SD and columns with the same lowercase letter are not significantly ($p < 0.05$) different

The total carotenoid contents at 20 ppt and 30 ppt salinity were very low with unchanged colored cell suspensions throughout the experimental period, which was similar to the bleached cell of *H. pluviaris* cultured in medium with 2% NaCl concentration (Sarada et al., 2002). It is possible that 0 ppt and 10 ppt salinity were optimal levels for *R. palustris*, which might be the essential factor for carotenoid synthesis, whereas 20 ppt and 30 ppt were extremely concentrated levels that probably inhibited carotenoid production because of excessive osmotic stress (Chen et al., 2006).

Dry cell weight

The mean ± SD dry cell weights at 0 ppt, 10 ppt, 20 ppt and 30 ppt salinity are given in Table 2 and ranged between 0.27 ± 0.08 g/L and 0.97 ± 0.34 g/L. The mean dry cell weight at 0 ppt salinity significantly increased during the first 6 d of culture (0.27 ± 0.07 g/L) to 27 d of culture (0.87 ± 0.42 g/L). At 10 ppt salinity, the mean dry cell weight was the highest at 18 d of culture (0.97 ± 0.34 g/L) and was significantly different from that at 6 d of culture (0.47 ± 0.26 g/L). The mean at 20 ppt salinity was high at 9 d of culture (0.59 ± 0.18 g/L), 18 d of culture (0.52 ± 0.20 g/L) and 27 d of culture (0.44 ± 0.08 g/L), but there were no significant differences. The mean dry cell weight at 30 ppt salinity continuously increased from 3 d of culture (0.45 ± 0.15 g/L) to 9 d of culture (0.70 ± 0.09 g/L) and then the mean dry cell weight decreased until 27 d of culture (0.62 ± 0.24 g/L). The lowest mean value was at 30 d of culture (0.31 ± 0.04 g/L), which was significantly different compared to 6 d, 9 d and 27 d of culture (0.64 ± 0.10 g/L, 0.70 ± 0.09 g/L and 0.62 ± 0.24 g/L, respectively).

Comparison of the mean dry cell weight among different salinity levels at different stages revealed that the dry cell weight for the four salinity levels were not significantly different during the first 15 d of culture and during 21–27 d of culture. However, the mean dry cell weight at 18 d of culture was significantly different between 10 ppt (0.97 ± 0.34 mg/L) and 30 ppt salinity (0.42 ± 0.06 mg/L). At 30 d of culture, the mean dry cell weight was higher at 10 ppt salinity (0.67 ± 0.12 mg/L) than that at 20 ppt and 30 ppt salinity (0.33 ± 0.07 mg/L and 0.31 ± 0.04 mg/L, respectively).

The results showed that the growth patterns of *R. palustris* at different salinity levels had similar fluctuations. Normally, *R. palustris* lives in mesophilic fresh water (Imhoff et al., 2005) such as lakes (Bianchi et al., 2010), wastewater (Vikineswary et al., 1997) and hot springs (Resnick and Madigan, 1989). It was assumed that *R. palustris* might be able to grow under a wide range of conditions because different levels of salinity (0–30 ppt) had no negative impact on its growth. Nunkaew et al. (2015) revealed that bacteria *R. palustris* strains secreted extracellular exopolymeric substances when it survived in a high NaCl concentration and these substances were also linked to sodium ions (Na^+) for salt stress mitigation.

Comparison of total carotenoid production and dry cell weight

The total carotenoids at 0 and 10 ppt salinity cultures gradually increased while the dry cell weight slightly changed in all cultures. There were low or none total carotenoids in the 20 and 30 ppt cultures, whereas the dry cell weight fluctuated every 9 d (Fig. 2). The cell growth patterns at 0 and 10 ppt salinity could be divided into three stages: 0–9 d, 9–18 d and after 18 d of culture. During the first stage, it was assumed that *R. palustris* cells grew and simultaneously accumulated carotenoids in their cells, which might have caused the the larger cell size. The second stage was the stationary phase, in which the death and growth rates were equal and so new cells might be fewer but larger in size than the cells in the first stage. Finally, the third stage was the death phase with a decline in the number of cells but cell size was largest because the cells of *R. palustris* accumulated the highest carotenoid content at both of 0 and 10 ppt salinity. Choi et al. (2011) found that the vegetative cells of *H. pluvialis* were larger than juvenile cells and these cells could accumulate greater quantities of astaxanthin.

Analysis of carotenoids

The carotenoid types were identified using spectrophotometric determination and the TLC method. The absorption spectra (Fig. 3A) of *R. palustris* cultured at 0 ppt salinity had five peaks at 364 nm, 479 nm, 602 nm, 690 nm and 775 nm, whereas at 10 ppt salinity there were four peaks at 364 nm, 474 nm, 602 nm and 773 nm. Both of these culture conditions presented a peak in the visible wavelength of carotenoids (400–490 nm) at 479 nm (at 0 ppt) and 474 nm (at 10 ppt). Additionally, their peak at 364 nm was in the ultraviolet area and 775 nm and 773 nm were in the near infrared region and the latter were probably bacteriochlorophyll a (770 nm) (Gottstein and Scheer, 1983). Wang et al. (2017) believed that the role of bacteriochlorophyll in resisting high salinity might be similar to that of carotenoids.

Table 2 Dry cell weight of *R. palustris* cultured at different salinity levels in *Rhodopseudomonas* medium during 30 d

Culture period (d)	Dry cell weight (g/L)			
	Salinity level (ppt)			
	0	10	20	30
3	0.27 ± 0.08^{a}	0.40 ± 0.05^{a}	0.45 ± 0.12^{a}	0.45 ± 0.15^{ab}
6	0.27 ± 0.07^{a}	0.47 ± 0.26^{a}	0.42 ± 0.10^{a}	0.64 ± 0.10^{a}
9	0.72 ± 0.12^{bc}	0.67 ± 0.27^{ab}	0.59 ± 0.18^{a}	0.70 ± 0.09^{a}
12	0.54 ± 0.13^{ab}	0.51 ± 0.34^{ab}	0.43 ± 0.17^{a}	0.53 ± 0.16^{ab}
15	0.73 ± 0.12^{bc}	0.53 ± 0.20^{ab}	0.47 ± 0.28^{a}	0.49 ± 0.03^{ab}
18	0.69 ± 0.23^{bc}	0.97 ± 0.34^{b}	0.52 ± 0.20^{a}	0.42 ± 0.06^{ab}
21	0.42 ± 0.23^{ab}	0.58 ± 0.27^{ab}	0.37 ± 0.19^{a}	0.56 ± 0.20^{ab}
24	0.47 ± 0.18^{ab}	0.60 ± 0.12^{ab}	0.39 ± 0.16^{a}	0.41 ± 0.10^{ab}
27	0.87 ± 0.42^{c}	0.62 ± 0.04^{ab}	0.44 ± 0.08^{a}	0.62 ± 0.24^{a}
30	0.49 ± 0.10^{ab}	0.67 ± 0.12^{ab}	0.33 ± 0.07^{a}	0.31 ± 0.04^{b}

mean values with different superscript letters within each column denote significant ($p < 0.05$) differences between groups.

The TLC analysis indicated several bands of carotenoid extraction (Fig. 3B) in the chromatogram, which were derived from *R. palustris* cultured at 0 and 10 ppt salinity. The carotenoid extraction focused on the yellow bands that showed retardation factor (R_f) values of 0.44 and 0.94 for 0 ppt salinity as well as 0.46 and 0.95 for 10 ppt salinity. In addition, the R_f values of the authentic astaxanthin and shrimp extraction were 0.43 based on standard usage and the yellow bands for 0 ppt salinity and for 10 ppt salinity were similar to those for authentic standard and shrimp extraction (0.43). Therefore, the bands could be identified as astaxanthin. The R_f values of 0.94 (0 ppt salinity) and 0.95 (10 ppt salinity) matched with those of β-carotene (0.95) (Quach et al., 2004).

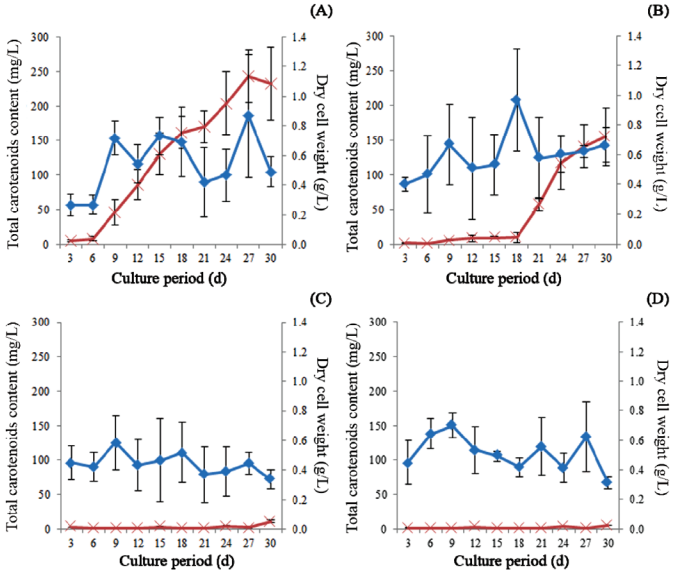

Fig. 2 Total carotenoid content (×) and dry cell weight (◆) accumulated in *R. palustris* at salinity levels of: (A) 0 parts per thousand (ppt); (B) 10 ppt; (C) 20 ppt; (D) 30 ppt, where values are means of triplicates and error bars show ± SD

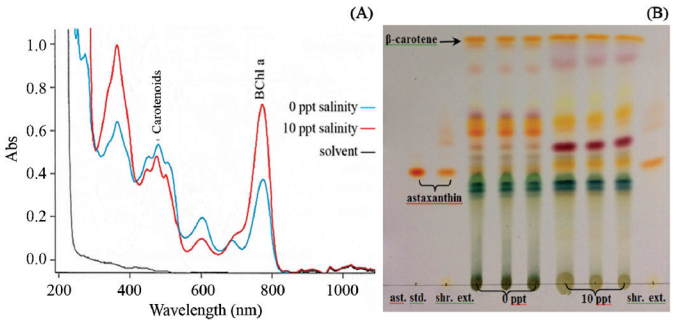

Fig. 3 (A) Absorption spectra of 0 parts per thousand (ppt) and 10 ppt salinity at 190–1100 nm, where BChl a = bacteriochlorophyll a; (B) thin-layer chromatography separation of carotenoid extracts from *R. palustris* at different salinity levels compared to astaxanthin standard (ast. std.) and shrimp extraction (shr. ext.)

Many researchers reported that *R. palustris* accumulated carotenoids such as lycopene, rhodopin and spirilloxanthin series (McDermott et al., 1973; Mangels et al., 1986; Mehrabi et al., 2001; Imhoff, 2005). However, the current results indicated that *R. palustris* cultured in medium with 0 ppt and 10 ppt salinity could synthesize some carotenoids that might be astaxanthin and β-carotene. Thus, the salinity factor could induce *R. palustris* to produce unusual carotenoids (Ide et al., 2012).

In conclusion, various salinity levels affected carotenoid production in *R. palustris* and 0 and 10 ppt salinity were the optimal levels that could stimulate *R. palustris* to synthesize carotenoids. However, 20 and 30 ppt salinity produced less or no carotenoids and there was unchanged color in the cell suspension after 30 d of culture. While different salinity levels had no negative impact on *R. palustris* culture, cell growth seemed to have a similar pattern at all four levels. The carotenoids at 0 ppt and 10 ppt salinity probably accumulated unusual kinds as β-carotene and astaxanthin. Thus, the effect of salinity on the cell weight and the intermediate carotenoid extract should be further investigated. Moreover, the mechanism of carotenoid and bacteriochlorophyll production is also of interest for future investigation.

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References

Aksu, Z., Eren, A.T. 2005. Carotenoids production by the yeast *Rhodotorula mucilaginosa*: use of agricultural wastes as a carbon source. Process Biochem. 40: 2985–2991.

Aksu, Z., Eren, A.T. 2007. Production of carotenoids by the isolated yeast of *Rhodotorula glutinis*. Biochem. Eng. J. 35: 107–113.

Alipour, S., Habibi, A., Taavoni, S., Varmira, K. 2017. β-carotene production from soap stock by loofa-immobilized *Rhodotorula rubra* in an airlift photobioreactor. Process Biochem. 54: 9–19.

Atlas, R.M. 2010. Handbook of Microbiological Media, 4th eds. CRC Press. Boca Raton, FL, USA.

Bianchi, L., Mannelli, F., Viti, C., Adessi, A., De Philippis, R. 2010. Hydrogen-producing purple non-sulfur bacteria isolated from the trophic lake Averno (Naples, Italy). Int. J. Hydrogen Energy. 35: 12216–12223.

Brown, S.R. 1968. Bacterial carotenoids from freshwater sediments. Limnol. Oceanogr. 13: 233–241.

Chandi, G.K., Gill, B.S. 2011. Production and characterization of microbial carotenoids as an alternative to synthetic colors: A review. Int. J. Food Prop. 14: 503–513.

Chen, D., Han, Y., Gu, Z. 2006. Application of statistical methodology to the optimization of fermentative medium for carotenoids production by *Rhodobacter sphaeroides*. Process Biochem. 41: 1773–1778.

Chien, Y.H., Shiau, W.C. 2005. The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus* Bate. J. Exp. Mar. Biol. Ecol. 318: 201–211.

Choi, Y.E., Yun, Y.S., Park, J.M., Yang, J.W. 2011. Determination of the time transferring cells for astaxanthin production considering two-stage process of *Haematococcus pluvialis* cultivation. Bioresource Technol. 102: 11249–11253.

Choudhari, S., Singhal, R. 2008. Media optimization for the production of β-carotene by *Blakeslea trispora*: A statistical approach. Bioresource Technol. 99: 722–730.

Globenewswire. 2013. Global carotenoids market expected to reach USD 1.52 billion in 2021. California, United State. https://goo.gl/YA3HSc/, 22 June 2017.

Gómez, P.I., Barriga, A., Cifuentes, A.S., González, M.A. 2003. Effect of salinity on the quantity and quality of carotenoids accumulated by *Dunaliella salina* (strain CONC-007) and *Dunaliella bardawil* (strain ATCC 30861) Chlorophyta. Biol. Res. 36: 185–192.

Gottstein, J., Scheer, H. 1983. Long-wavelength-absorbing forms of bacteriochlorophyll a in solutions of Triton X-100. Proc. Natl. Acad. Sci. USA. 80: 2231–2234.

Homthong, S. 1998. Production of astaxanthin from *Haematococcus* sp. M.Sc. thesis, Faculty of Science, Kasetsart University. Bangkok, Thailand.

Hu, Z.C., Zheng, Y.G., Wang, Z., Shen, Y.C. 2006. pH control strategy in astaxanthin fermentation bioprocess by *Xanthophyllomyces dendrorhous*. Enzyme Microb. Tech. 39: 586–590.

Ide, T., Hoya, M., Tanaka, T., Harayama, S. 2012. Enhanced production of astaxanthin in *Paracoccus* sp. strain N-81106 by using random mutagenesis and genetic engineering. Biochem. Eng. J. 65: 37–43.

Imhoff, J.F. 2005. Genus IX. *Rhodopseudomonas*. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds.). Bergey's Manual of Systematic Bacteriology, Vol. 2: The Proteobacteria, Part C The Alpha-, Beta-, Delta-, and Epsilon Proteobacteria, 2nd ed. Springer Science+Business Media, Inc. New York, NY, USA, pp. 473-474.

Imhoff, J.F., Hiraishi, A., Süling, J. 2005. Anoxygenic phototrophic purple bacteria. In: Brenner, D.J., Krieg, N.R., Staley, J.T., Garrity, G.M. (Eds.). Bergey's Manual of Systematic Bacteriology, Vol. 2: The Proteobacteria, Part A Introductory Essays. 2nd ed. Springer Science+Business Media, Inc. New York, NY, USA, pp. 119-132.

Kobayashi, M., Katsuragi, T., Tani, Y. 2001. Enlarged and astaxanthin-accumulating cyst cells of the green alga *Haematococcus pluvialis*. J. Biosci. Bioeng. 92: 565–568.

Kuo, F.S., Chien, Y.H., Chen, C.J. 2012. Effects of light sources on growth and carotenoid content of photosynthetic bacteria *Rhodopseudomonas palustris*. Bioresource Technol. 113: 315–318.

Malisorn, C., Suntornsuk, W. 2008. Optimization of β-carotene production by *Rhodotorula glutinis* DM28 in fermented radish brine. Bioresource Technol. 99: 2281–2287.

Mangels, L.A., Favinger, J.L., Madigan, M.T., Gest, H. 1986. Isolation and characterization of the N_2-fixing marine photosynthetic bacterium *Rhodopseudomonas marina*, variety *agilis*. FEMS Microbiol. Lett. 36: 99–104.

McDermott, J.C.B., Ben-Aziz, A., Singh, R.K., Britton, G., Goodwin, T.W. 1973. Recent studies of carotenoid biosynthesis in bacteria. Pure Appl. Chem. 35: 29–46.

Mehrabi, S., Ekanemesang, U.M., Aikhionbare, F.O., Kimbro, K.S., Bender, J. 2001. Identification and characterization of *Rhodopseudomonas* spp., a purple, non-sulfur bacterium from microbial mats. Biomol. Eng. 18: 49–56.

Morowvat, M.H., Ghasemi, Y. 2016. Culture medium optimization for enhanced β-carotene and biomass production by *Dunaliella salina* in mixotrophic culture. Biocatal. Agric. Biotechnol. 7: 217–223.

Niu, J., Wen, H., Li, C.H., Liu, Y.J., Tian, L.X., Chen, X., Huang, Z., Lin, H.Z. 2014. Comparison effect of dietary astaxanthin and β-carotene in the presence and absence of cholesterol supplementation on growth performance, antioxidant capacity and gene expression of *Penaeus monodon* under normoxia and hypoxia condition. Aquaculture 422–423: 8–17.

Nunkaew, T., Kantachote, D., Nitoda, T., Kanzaki, H. 2015. Characterization of exopolymeric substances from selected *Rhodopseudomonas palustris* strains and their ability to adsorb sodium ions. Carbohyd. Polym. 115: 334–341.

Paliwal, C., Pancha, I., Ghosh, T., Maurya, R., Chokshi, K., Bharadwaj, S.V.V., Ram, S., Mishra, S. 2015. Selective carotenoid accumulation by varying nutrient media and salinity in *Synechocystis* sp. CCNM 2501. Bioresour. Technol. 197: 363–368.

Qin, S., Liu, G.X., Hu, Z.Y. 2008. The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (Chlorophyceae). Process Biochem. 43: 795–802.

Quach, H.T., Steeper, R.L., Griffin, G.W. 2004. An improved method for the extraction and thin-layer chromatography of chlorophyll a and b from spinach. J. Chem. Educ. 81: 385–387.

Resnick, S.M., Madigan, M.T. 1989. Isolation and characterization of a mildly thermophilic non sulfur purple bacterium containing bacteriochlorophyll b. FEMS Microbiol. Lett. 65: 165–170.

Sanchez, S., Ruiz, B., Rodríguez-Sanoja, R. 2013. Microbial production of food ingredients, enzymes and nutraceuticals. In: McNeil, B., Archer, D., Giavasis, I., Harvey, L. (Eds.). Microbial Production of Food Ingredients, Enzymes and Nutraceuticals. Woodhead Publishing Limited, Cambridge, UK, pp.194–233.

Sarada, R., Tripathi, U., Ravishankar, G.A. 2002. Influence of stress on astaxanthin production in *Haematococcus pluvialis* grown under different culture conditions. Process Biochem. 37: 623–627.

Thanomchaisanit, P. 2013. Production of astaxanthin from bacteria *Rhodopseudomonas* sp. M.Sc. thesis, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

Vikineswary, S., Getha, K., Maheswary, S., Chong, V.C., Shaliza, I., Sastry, C.A. 1997. Growth of *Rhodopseudomonas palustris* strain B 1 in sago starch processing wastewater. In: Wise, D.L. (Ed.). Global Environmental Biotechnology: Proceedings of the Third Biennial Meeting of the International Society for Environmental Biotechnology. Boston, MA, USA, pp. 335–348.

Wang, H, Yang, A., Zhang, G., Ma, B., Meng, F., Peng, M., Wang, H. 2017. Enhancement of carotenoid and bacteriochlorophyll by high salinity stress in photosynthetic bacteria. Int. Biodeter. Biodegr. 121: 91–96.

Yokoyama, A., Miki, W. 1995. Composition and presumed biosynthetic pathway of carotenoids in the astaxanthin-producing bacterium *Agrobacterium aurantiacum*. FEMS Microbiol. Lett. 128: 139–144.