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Effect of Culture Conditions on Canthaxanthin Production by *Dietzia natronolimnaea* HS-1

KHODAIYAN, FARAMARZ, SEYED HADI RAZAVI*, ZAHRA EMAM-DJOMEH, SEYED MOHAMMAD ALI MOUSAVI, AND MOHAMMAD AMIN HEJAZI[1]

Department of Food Science and Engineering, Faculty of Biosystem Engineering, University of Tehran, Tehran, Iran
[1]Department of Microorganism & Biosafety, Agricultural Biotechnology Research Institute of Iran (ABRII), Karaj, Iran

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Abstract This study investigated the effects of various culture parameters (carbon sources, temperature, initial pH of culture, NaCl concentration, and light) on the growth and canthaxanthin production by *Dietzia natronolimnaea* HS-1. The results showed that the most effective carbon source for growth and canthaxantin production was glucose, and the best pH and temperature were 7 and 31°C, respectively. In addition, the biomass and canthaxanthin production increased in a medium without NaCl and in the presence of light. Under the optimized conditions, the maximum biomass, total carotenoid, and canthaxanthin production were 6.12±0.21 g/l, 4.51±0.20 mg/l, and 4.28±0.15 mg/l, respectively, in an Erlenmeyer flask system, yet increased to 7.25 g/l, 5.48 mg/l, and 5.29 mg/l, respectively, in a batch fermenter system.

Key words: Carotenoid, canthaxanthin, culture conditions, *Dietzia natronolimnaea* HS-1, fermenter

Canthaxanthin (4, 4'-diketo-β-carotene) is a ketocarotenoid found in certain animals, plants, and microorganisms [7], plus it is responsible for the orange-red color of egg yolk and the flesh of many marine animals [26]. Thus, because of its color and strong antioxidant activity, canthaxanthin is widely applied in the medical [7], pharmaceutical [7], cosmetic [14], poultry [27], fishery [27], and food industries [16].

At present, the large market for carotenoids is satisfied through chemical synthesis, although this has various disadvantages, as the chemical synthesis of carotenoids requires a very high level of control and can produce compounds that have undesired side effects and may be allergens in certain consumers. Therefore, in view of the global economic value of carotenoids and increasing awareness of consumers, the production of these materials from natural sources has become an area of intensive investigation [6].

For the industrial production of carotenoids, microorganisms are preferred over other natural sources, such as vegetables and fruits, owing to problems of seasonal and geographic variability in production. In addition, there are economic advantages to microbial processes that use agricultural waste and industrial wastewater as substrates [11]. Nonetheless, despite the importance of the microbial production of these compounds, relatively few microorganisms have been identified as suitable for commercial applications of ketocarotenoids, where these microorganisms include the yeast *Phaffia rhodozyma* [20, 21], fresh-water green alga *Haematococcus pluvialis* [23], and green alga *Chlorococcum* sp. strain MA-1 [34], all of which accumulate astaxanthin. However, very little data are available on the commercial production of canthaxanthin by microorganisms [7]. Thus, the search continues for microbial sources for canthaxanthin production and the optimum conditions for the production of these compounds.

The bacterium *Dietzia natronolimnaea* is Gram positive, catalase positive, and oxidase negative with orange colonies [13], and *D. natronolimnaea* HS-1 was isolated during a routine screening of pigmented microorganisms. In preliminary experiments, the main pigment of this strain was identified to be a canthaxanthin [30], and therefore, the main purpose of the present study was to introduce *Dietzia natronolimnaea* HS-1 as a new canthaxanthin-producing bacterium and optimize the effects of different growth medium factors on canthaxanthin production.

*Corresponding author
Phone: 98-261-2248804; Fax: 98-261-2248804;
E-mail: srazavi@ut.ac.ir

MATERIALS AND METHODS

Materials

The glucose, peptone, malt extract, yeast extract, sugars, NaCl salt, agar, and Antifoam 289 were all obtained from the Sigma-Aldrich Chemical Company (U.S.A.). The pure ethanol (99.9%) was purchased from the Bidestan Company (Iran), the canthaxanthin standard supplied by Dr. Ehrenstorfer GmbH (Germany), and the acetonitrile and methanol were of HPLC grade from Merck (Germany).

Microorganism

The strain *D. natronolimnaea* HS-1 (DSM 44860) was isolated in the Laboratoire des Science du Génie Chimique by Razavi [30], and maintained on yeast/malt (YM) agar plates containing (per liter): 10 g glucose, 5 g peptone, 3 g malt extract, 3 g yeast extract, and 15 g agar. Single colonies were transferred to a fresh plate every month, incubated for 4 days, and thereafter kept under refrigeration at 4°C.

Preparation of Inoculum

A pure culture of *D. natronolimnaea* HS-1 from the YM agar was transferred into 500-ml Erlenmeyer flasks containing 100 ml of a GPY medium (per liter: 10 g glucose, 10 g peptone, 6 g yeast extract), incubated in a rotary shaker (180 rpm) at 28±1°C, and after 72 h used as the inoculum.

Culture Conditions

Ten ml of the inoculum was transferred into 500-ml Erlenmeyer flasks containing 100 ml of the GPY medium. The flasks were then incubated in a rotary shaker (180 rpm) under constant illumination (600±50 lux) provided by cool white fluorescent lamps (30 W, Resell-Shark, Switzerland). Subsamples were periodically harvested during an 8-day period. To study the effect of light on growth and carotenogenesis, some of the Erlenmeyer flasks were covered with aluminum foil to make them impermeable to light.

Using the optimum conditions determined by the Erlenmeyer flask system (temperature, 31°C; pH, 7; illumination, 600±50 lux), batch fermenter cultures were then carried out using a 3-l fermenter (Bio Flo 2000 fermenter, model BF-2000, New Brunswick Scientific Co., Edison, N.J., U.S.A.) containing 1.8 l of the above-mentioned GPY medium. A pH of 7 was maintained during the fermentation culture using NaOH (2 M) and HCl (2 M). In additon, aerobic conditions were created inside the fermenter by flushing air until the concentration of dissolved oxygen reached 75% based on an aeration rate of 3 vvm and agitation of 300–400 rpm. The foam was controlled by adding antifoam 289, and the fermenter inoculated with 10% preculture. At given cultivation times, samples were withdrawn from the fermenter to determine the rate of growth and canthaxanthin production.

Dry Weight and Glucose Measurement

The biomass dry weight was determined by harvesting 5-ml culture samples, filtering the cells through a 0.2-μm filter (Sigma-Aldrich Co., U.S.A.) (dried at 65°C for 4 h), washing the cells with distilled water, and drying them at 105°C to a constant weight (48 h). Meanwhile, the glucose was measured using an HPLC method.

Extraction and Analysis of Carotenoids

Ten-ml aliquots were centrifuged at 5,000 ×*g* for 10 min at 4°C. The pellets were then washed twice with a solution of 9 g/l NaCl and centrifuged again. Next, the supernatant was resuspended in 3 ml of pure ethanol by vortexing for 5 min, and the pellets centrifuged again to extract the pigment. This was repeated three times. Thereafter, the pigments were completely extracted using a water bath (45°C), and the carotenoid extracts subsequently filtered through a 0.2-μm hydrophobic fluorophore membrane (Sigma-Aldrich Co., U.S.A.) and analyzed by scanning the absorbance of the wavelength spectra of 300–600 nm using a spectrophotometer (UV-Visible, Cary 300, Varians Co., Germany). The maximum absorbance was determined at a wavelength of 474 nm, which conformed with standard canthaxanthin λ_{max}. The total carotenoid concentration was calculated following the formula provided by An *et al.* [2], and the concentrations of individual carotenoids determined according to the modified method of Razavi *et al.* [29], using an HPLC (Knauer, Germany) equipped with a UV-visible detector (K-2600, Knauer, Germany) and pump (K-1001, Knauer, Germany). The chromatographic separation was performed on a Nucleosil 100 C18, 5.0 μm (125× 4.0 mm), where the temperature of the column was maintained at room temperature and the mobile phase was acetonitrile:methanol (80:20, V/V) at a flow rate of 1 ml/min. The eluant was monitored at 480 nm. To protect the column, a pre-column (5×4.0 mm) of the same material was also used. The volume of the injected solutions was 100 μl.

Data Analyses

The effects of different growth medium factors were optimized for the maximum production of canthaxanthin using the traditional "one-factor-at-a-time" (OFAT) design. The experiments with the Erlenmeyer flask system were performed based on four replications, and Duncan's test used to compare the data. The data are expressed as the mean±standard error of the mean (SEM). The statistical analyses were performed using SPSS 10 software (SPSS Science, Birmingham, U.K.). In addition, Microsoft Excel 7 software (Microsoft, Redmond, U.S.A.) was used to plot the curves.

Table 1. Effect of carbon sources on growth and canthaxanthin production by *D. natronolimnaea* HS-1. Growth medium conditions (g/l): carbon source (10), peptone (10), yeast extract (6); initial pH: 7; temperature: 28°C; in rotary shaker (180 rpm); cultivation period: 8 days.

Carbon sources	μ_{max} (h^{-1})	Biomass (g/l)	Carotenoid (mg/l)	Carotenoid (mg/g)	Canthaxanthin (mg/l)
Glucose	0.035±0.003^{d}	5.87±0.20^{g}	4.10±0.21^{h}	0.70±0.06^{de}	4.01±0.23^{g}
Fructose	0.030±0.002^{cd}	4.90±0.23^{f}	4.00±0.28^{g}	0.82±0.09^{e}	3.54±0.21^{f}
Mannose	0.027±0.003^{bc}	3.81±0.18^{d}	2.29±0.19^{e}	0.60±0.03^{c}	2.00±0.15^{d}
Galactose	0.016±0.004^{a}	1.95±0.13^{a}	0.80±0.12^{ab}	0.41±0.07^{ab}	0.76±0.1^{ab}
Sucrose	0.027±0.005^{bc}	4.34±0.32^{e}	2.87±0.25^{f}	0.66±0.05^{cd}	2.45±0.19^{e}
Lactose	0.015±0.004^{a}	1.91±0.13^{a}	1.18±0.15^{c}	0.62±0.03	0.99±0.15^{bc}
Maltose	0.015±0.003^{a}	1.78±0.14^{a}	0.96±0.11^{abc}	0.54±0.10^{bc}	0.84±0.17^{b}
D-Xylose	0.019±0.001^{a}	2.22±0.11^{b}	0.74±0.21^{ab}	0.33±0.08^{a}	0.70±0.20^{ab}
Sorbitol	0.014±0.005^{a}	1.81±0.12^{a}	0.69±0.17^{a}	0.38±0.01^{a}	0.52±0.14^{a}
Mannitol	0.013±0.006^{a}	1.75±0.10^{a}	0.65±0.20^{a}	0.37±0.05^{ab}	0.61±0.19^{ab}
Raffinose	0.017±0.001^{a}	1.94±0.13^{a}	0.89±0.21^{abc}	0.46±0.06^{ab}	0.80±0.29^{abc}
Glycerol	0.024±0.002^{b}	2.29±0.11^{b}	1.10±0.22^{bc}	0.48±0.06^{b}	1.04±0.25^{bc}
Dextrin	0.025±0.004^{bc}	3.04±0.13^{c}	1.67±0.19^{d}	0.55±0.07^{bc}	1.34±0.25^{c}

RESULTS

In preliminary experiments by Razavi [30], the best nitrogen source for canthaxanthin production by *D. natronolimnaea* HS-1 was found to be a composition of 10 g/l peptone and 6 g/l yeast extract (data not shown). Therefore, this compound was used in all the experiments.

Effect of Carbon Sources on Biomass and Canthaxanthin Production

When investigating the effects of various carbon sources on cell growth and canthaxanthin production, the highest level was observed in the presence of glucose and fructose, although sucrose and mannose were also found to have a significant effect on carotenoid and canthaxanthin production. However, for the top two carbon sources, glucose and fructose, significant differences were found between the values for their biomass, total carotenoid, and canthaxanthin production (Table 1). Therefore, among the 13 tested carbon sources, glucose was identified as the best source for canthaxanthin production (4.01±0.23).

Effect of Different Temperatures on Biomass and Canthaxanthin Production

To study the effect of different temperatures (13, 20, 28, 31, 34, 40, and 50°C) on the maximum specific growth rate (μ_{max}), biomass production, and carotenogenesis, samples were periodically taken and the values for the biomass, total carotenoid, and canthaxanthin production reported for the time showing the maximum biomass. *D. natronolimnaea* HS-1 exhibited a maximum growth rate of 0.046±0.003 h^{-1} at 31°C. No growth was observed at 50°C, and an approximately two-fold decrease in growth rate was observed when *D. natronolimnaea* HS-1 was cultured at 13°C in contrast to incubation at 31°C. The highest values for biomass and canthaxanthin production were found at 31°C (6.09±0.19 g/l, 4.06±0.16 mg/l, respectively). As indicated in Table 2, the carotenoid accumulation (mg/g) increased when the temperature was decreased from 31°C to 13°C. An approximately 40.6% increase in carotenoid synthesis was observed when *D. natronolimnaea* HS-1 was cultured at 13°C instead of 31°C.

Table 2. Effect of different temperatures on growth and canthaxanthin production by *D. natronolimnaea* HS-1. Growth medium conditions (g/l): glucose (10), peptone (10), yeast extract (6); initial pH: 7; in rotary shaker (180 rpm).

Temperature (°C)	μ_{max} (h^{-1})	$Biomass_{max}$ (g/l)	Time (h) $Biomass_{max}$	Carotenoid (mg/l)	Carotenoid (mg/g)	Canthaxanthin (mg/l)
13	0.016±0.001^{a}	5.91±0.18^{b}	340	5.73±0.21^{e}	0.97±0.08^{d}	3.82±0.17^{bc}
20	0.026±0.003^{b}	6.01±0.21^{b}	264	5.04±0.23^{d}	0.84±0.04^{c}	3.78±0.18^{bc}
28	0.035±0.002^{d}	5.90±0.26^{b}	192	4.12±0.16^{c}	0.70±0.07^{b}	3.75±0.13^{b}
31	0.046±0.003^{e}	6.09±0.19^{b}	168	4.23±0.20^{c}	0.69±0.06^{b}	4.06±0.16^{c}
34	0.030±0.002^{c}	5.76±0.20^{b}	168	3.71±0.21^{b}	0.64±0.05^{b}	3.53±0.15^{b}
40	0.018±0.001^{a}	1.94±0.14^{a}	192	0.75±0.14^{a}	0.35±0.04^{a}	0.68±0.06^{a}

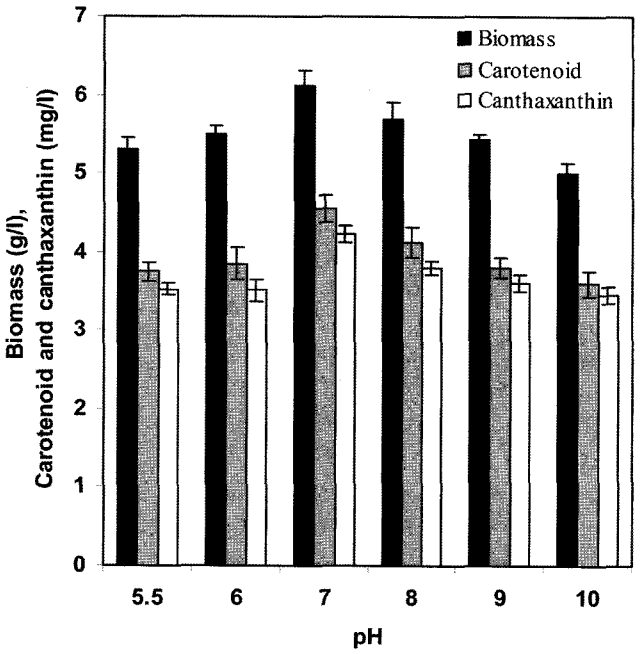

Fig. 1. Effect of initial pH of growth medium on biomass and canthaxanthin production by *D. natronolimnaea* HS-1.

Effect of Initial pH of Growth Medium on Biomass and Canthaxanthin Production

When increasing the pH, the growth and carotenoid production also increased and reached a maximum level at pH 7.0, and then decreased at higher pH values (Fig. 1). Moreover, the optimal pH value of 7.0 yielded the highest rates of biomass (6.09±0.18 g/l), carotenoid (4.47±0.18 mg/l), and canthaxanthin (4.26±0.11 mg/l) production.

Effect of NaCl Concentration on Biomass and Canthaxanthin Production

To determine the effect of the NaCl concentration on biomass and carotenoid production, media containing 0–100 g/l NaCl were used. When increasing the concentration of NaCl in the growth medium, the biomass and pigment production both decreased, as shown in Fig. 2. The figure also shows that the bacterial strain was halotolerant, and acceptable amounts of carotenoid and canthaxanthin were produced with all NaCl concentrations less than 50 g/l.

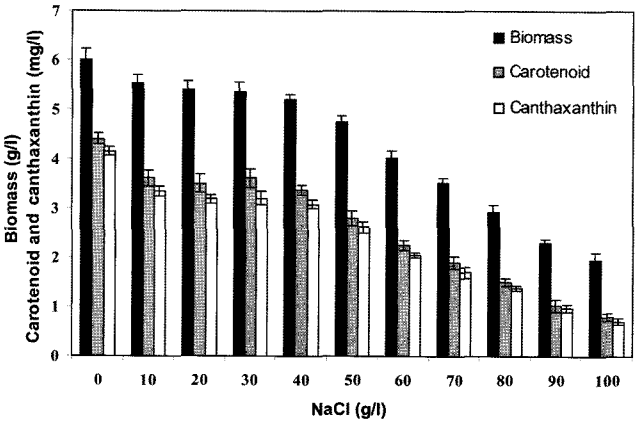

Fig. 2. Effect of NaCl concentration of culture medium on biomass and canthaxanthin production by *D. natronolimnaea* HS-1.

Table 3. Effect of light on growth and canthaxanthin production by *D. natronolimnaea* HS-1; growth medium conditions (g/l): glucose (10), peptone (10), yeast extract (6), NaCl (0); temperature: 31°C; initial pH: 7; in rotary shaker (180 rpm).

Condition	Biomass (g/l)	Carotenoid (mg/l)	Carotenoid (mg/g)	Canthaxanthin (mg/l)
Light	6.1±0.21[b]	4.5±0.20[b]	0.74±0.02[b]	4.28±0.15[b]
Dark	5.4±0.22[a]	3.7±0.20[a]	0.68±001[a]	3.48±0.12[a]

Effect of Light on Biomass and Canthaxanthin Production

To investigate the effect of light on cell growth and pigment formation, *D. natronolimnaea* HS-1 was grown under dark and illuminated conditions. Table 3 shows that cultivation of the bacterium in the absence of light decreased its cell growth and pigment formation. In addition, there were significant decreases in the biomass, carotenoid, and canthaxanthin production (11.5%, 17.8%, and 18.7%, respectively).

Fig. 3 shows the time-course profile of the production of cells and canthaxanthin by *D. natronolimnaea* HS-1 under the determined optimum conditions, where the maximum biomass (6.12±0.21 g/l) production was on day 7, while the maximum canthaxanthin (4.28±0.15 mg/l) production occurred on day 8.

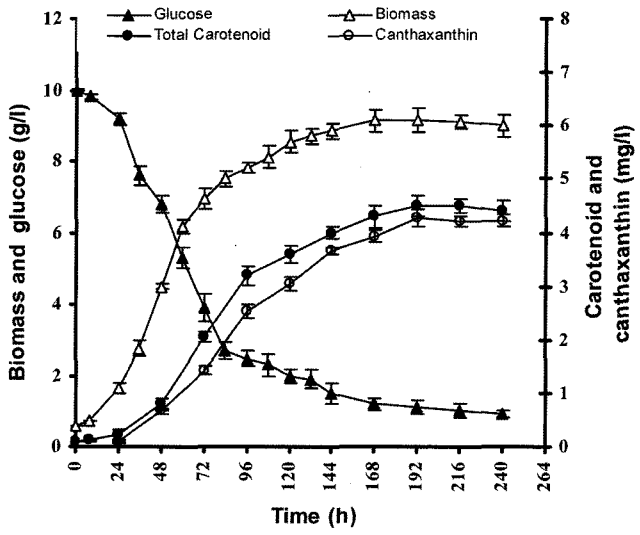

Fig. 3. Time-course profile of production of biomass, total carotenoid, and canthaxanthin by *D. natronolimnaea* HS-1 in an Erlenmeyer flask system under optimum conditions.

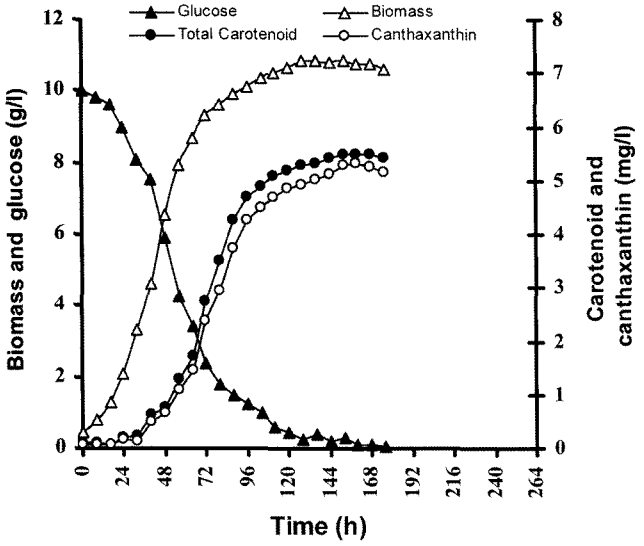

Fig. 4. Time-course profile of production of biomass, total carotenoid, and canthaxanthin by *D. natronolimnaea* HS-1 in a batch fermenter system under optimum conditions determined by an Erlenmeyer flask system.

Cell Growth and Canthaxanthin Production Kinetics in Batch Fermenter

To study the cell growth and canthaxanthin production kinetics in a batch fermenter, *D. natronolimnaea* HS-1 was cultivated under the optimum conditions determined by the Erlenmeyer flask system [glucose (10 g/l), growth medium without NaCl, temperature (31°C), pH (7), and light (600±50 lux)]. Fig. 4 shows that the maximum biomass production (7.25 g/l) was obtained after 128 h, while the maximum total carotenoid (5.48 mg/l) and canthaxanthin (5.29 mg/l) production were observed after 152 h, namely 24 h after the beginning of the stationary phase. The maximum growth rate in this system was 0.056 h^{-1}.

DISCUSSION

Ketocarotenoids like astaxanthin and canthaxanthin are extensively used in many industries owing to their vital and multifunctional roles in photoprotection, color pigments, and strong antioxidant properties [7]. The high commercial demand for these carotenoids has long been supplied by chemical synthesis technology, but some of the byproducts resulting from certain chemical processes have undesirable side effects on consumers. Therefore, production from biological sources has developed as an important area of research over the last decade [6], including the identification of microbial sources for carotenoid (such as canthaxanthin) production, and determining the optimum conditions for the production of these compounds.

Accordingly, this study investigated the effects of various factors, such as carbon sources, temperature, initial pH of the growth media, NaCl concentration, and light, on biomass, total carotenoid, and canthaxanthin production using *Dietzia natronolimnaea* HS-1 as a new canthaxanthin-containing bacterium. These factors were optimized for the highest level of canthaxanthin production using the traditional "one-factor-at-a-time" (OFAT) design, where the experimental design involved the step-by-step improvement of each factor in an individual and nonsimultaneous manner, while maintaining the other factors at constant values [25].

In the present study, the most effective carbon source for biomass production and carotenogenesis was glucose, which led to the highest rates of canthaxanthin formation. Other important sugars for total carotenoid production were ranked as follows: fructose>sucrose>mannose>dextrin>lactose>glycerol (Table 1).

Temperature is one of the most important environmental factors affecting the growth of microorganisms, and causes changes in many biosynthetic pathways, such as carotenoid biosynthesis [8]. In the present study, *D. natronolimnaea* HS-1 grew well at temperatures between 13 to 40°C. The maximum specific growth rate (μ_{max}) and highest biomass and canthaxanthin production occurred at 31°C. Thus, owing to the higher growth rate and lower time of fermentation (Table 2) at 31°C when compared with other temperatures, the optimum temperature for canthaxanthin production by *D. natronolimnaea* HS-1 was determined as 31°C. Similar results were also previously reported for *Gordonia jacobaea* MV-1 (a canthaxanthin producer) [12] and *R. mucilaginosa* [1].

With *D. natronolimnaea* HS-1, the highest level of carotenoid accumulation was found at lower temperatures, with the maximum pigmentation of 0.97±0.08 mg/g at 13°C (the lowest temperature tested). This observation is also in accordance with the previous findings of Fong and *et al.* [15], who attributed their results to the concept of an adaptive role of carotenoids in membrane stabilization under stress conditions of low temperatures. Carotenoids may play an important role in the regulation of membrane fluidity in this bacterium, thereby compensating for the decreased functionality of the biomembranes under cold stress conditions, as described in previous reports on *M. roseus* [18], *A. agilis* MB813 [15], and *Sporobolomyces ruberrimus* H110 [28]. It has also been suggested that the survival of certain microorganisms at low temperatures may increase because of the ability of carotenoids to rigidify membranes [31].

In addition to growth, the biosynthetic activity of the bacterium was also affected by the initial pH of the growth medium [1]. In the current study, an initial pH of 7 resulted in the highest levels of biomass and canthaxanthin. The same pH was also already reported to produce the maximum biomass and carotenoid production with *Rhodotorula mucilaginosa* [1] and extremely halophilic bacteria [5].

Increasing the concentration of NaCl in the growth medium resulted in decreased pigment production by *D. natronolimnaea* HS-1. Thus, the highest biomass, total

Table 4. Microbial sources of canthaxanthin.

Microorganism	Biomass (g/l)	Canthaxanthin (mg/l)	Canthaxanthin (mg/g)	Reference
Gordonia jacobaea MV-1	3.2	0.73	0.227	[12]
Chlorella emersonii	–	0.6	–	[7]
Haloferax alexandrinus TM[a]	3.17	2.19	0.69	[4]
Haloferax alexandrinus TM[b]	3.12	2.16	0.69	[4]
Micrococcus roseus	–	1.7	–	[7]
Halobacterium sp.	–	–	0.70	[5]
Bradyrhizobium sp.	0.58	0.78	1.34	[22]
Dietzia natronolimnaea HS-1[a]	7.25	5.31	0.73	This work
Dietzia natronolimnaea HS-1[b]	6.12	4.28	0.70	This work
Brevibacterium KY-4313	3–3.5	1–2	0.29–0.67	[26]
Brevibacterium KY-4313 (Mutant Strain)	12.6	9.3	0.74	[27]

[a]Cultivation in batch fermenter system.
[b]Cultivation in Erlenmeyer flask system.

carotenoid, and canthaxanthin production were found in a medium without NaCl. Nonetheless, *D. natronolimnaea* HS-1 was proven to be a salt-tolerant strain that was still able to produce considerable amounts of carotenoid in NaCl concentrations of less than 50 g/l. Therefore, given its NaCl tolerance and ability to use carbon sources like sucrose and lactose, *D. natronolimnaea* HS-1 is clearly a very promising source that can be used with agricultural and industrial waste, like molasses (a source of sucrose) and whey (a source of lactose), for the economical production of carotenoids. The effect of salt would seem to depend on the type of bacterium, as different species exhibit distinct patterns of biomass and carotenoid production in response to an increase in the medium salinity.

Irrespective of the intensity or duration of illumination, light irradiation positively affects the production and accumulation of carotenoids [8], and this increased carotenoid biosynthesis has been attributed to the effect of light on microorganism growth, meaning an increased bacterial biomass will improve the volumetric production of carotenoids (mg/l) [8]. Furthermore, the direct effect of light on the activity of the enzymes involved in carotenoid biosynthesis has also been suggested to increase the intracellular accumulation (mg/g) of carotenoids [8]. In the present study, the mass and volumetric production of the carotenoid pigments both increased significantly under illuminated conditions (Table 3). Illumination produced similar increases in carotenoid (zeaxanthin) production by *Flavobacterium* sp. [3], plus studies with other microorganisms, like yeasts, *Rhodotorula glutinis* [9], and *Phaffia rhodozyma* [32], have also confirmed the positive effect of this environmental factor on the biomass production and biosynthesis of carotenoids.

When investigating the total carotenoid and canthaxanthin production kinetics of *D. natronolimnaea* HS-1, the Erlenmeyer flask system and batch fermenter system both revealed that the maximum total carotenoid and canthaxanthin production were reached one day after the beginning of the stationary phase. Similar behavior has also been reported with other microorganisms, such as *Gordonia jacobaea* [33], *Flavobacterium* sp. [24], and *Rhodotorula glutinis* [10]. Yet, in contrast to *D. natronolimnaea* HS-1, the maximum total carotenoid formation in certain microorganisms, such as *Brevibacterium linens* [17] and extremely halophilic bacteria [5], occurs synchronous to their maximum biomass production.

When applying the optimized conditions (obtained from the Erlenmeyer flask system) to a batch fermenter, the maximum biomass, total carotenoid, and canthaxanthin production increased up to 21.8%, 44.4%, and 43.9%, respectively, compared with that produced with the Erlenmeyer flask system. The improved results observed in the batch fermenter were probably due to the increased availability of oxygen and constant pH in the growth media.

The amount of canthaxanthin produced under the optimized conditions represented more than 90% of the total carotenoid produced with each system.

In conclusion, a comparison with other important wild canthaxanthin-producing strains (Table 4) revealed that *D. natronolimnaea* HS-1 had the highest canthaxanthin production, making it a very promising source for the mass production of canthaxanthin. However, extended studies of different mutants with higher productivity and the genes of enzymes associated with the canthaxanthin biosynthetic pathway are also needed to improve the canthaxanthin production of *D. natronolimnaea* HS-1.

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