Algal Research 29 (2018) 41–48

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae *Phaeodactylum tricornutum*

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ARTICLE INFO

Keywords:
Fucoxanthin
Photo-bioreactor
Microalgae
Phaeodactylum tricornutum

ABSTRACT

Fucoxanthin is a carotenoid pigment produced by algae that has a range of potential health benefits. Despite the obvious interest in developing a process for the production of fucoxanthin, relatively few authors have systematically examined the impact of culture conditions (i.e. the light intensity, medium composition and CO_2 addition) on fucoxanthin production. In this work, we have addressed this issue using the marine microalgae *Phaeodactylum tricornutum*. It was found that at low light intensities (100 μmol photons $m^{-2} s^{-1}$) the specific fucoxanthin concentration was greater (42.8 ± 19.5 mg g^{-1}) than at a higher intensity of 210 μmol photons $m^{-2} s^{-1}$ (9.9 ± 4.2 mg g^{-1}). Addition of nitrate to the medium led to a significant increase in the specific fucoxanthin concentration with the maximum specific concentration (59.2 ± 22.8 mg g^{-1}), volumetric concentration (20.5 mg L^{-1}) and bioreactor productivity (2.3 mg L^{-1} day^{-1}) being observed with the nitrate enriched medium. These reproducible results from our systematic investigation into the effect of culture conditions on fucoxanthin production are highly encouraging and clearly demonstrate the potential for *P.tricornutum* to be employed as a natural source of fucoxanthin in nutraceutical applications.

1. Introduction

Microalgae are increasingly being seen as a promising source of high-value compounds for the food and nutraceutical industries. Examples of compounds that can be produced using microalgae include omega-3 fatty acids, polysaccharides and carotenoid pigments [1–4] with a wide range of the latter being produced including β-carotene, lutein, astaxanthin, peridinin and fucoxanthin [2,5].

In addition to their common application as colouring agents, the carotenoid pigments produced by algae also have a range of potential health benefits [2,6,7]. In particular, fucoxanthin and its metabolites have been shown [8,9] to exhibit diverse benefits including anti-oxidant, anti-inflammatory, anti-cancer and anti-obesity effects. Maeda et al. [10] demonstrated that addition of fucoxanthin to the diet of mice and rats led to a reduction in their abdominal white adipose tissue, while Abidov et al. [11] found that consumption of a mixture of pomegranate seed oil and extracts from brown seaweed containing fucoxanthin led to a statistically significant reduction in body weight for obese premenopausal women during a 16 week clinical trial. Given these indicative health benefits, there is clear potential for fucoxanthin to be examined more comprehensively as a nutraceutical compound.

Fucoxanthin is produced by both micro- and macroalgae of the divisions Heterokontophyta, Haptophyta and Dinophyta [12,13]. However, typical concentrations found in microalgae are at least an order of magnitude higher than those in macroalgae, where the fucoxanthin concentration is generally < 1 mg g^{-1} [14,15], and hence microalgae are seen as the more promising source. A summary of previously published microalgae studies is presented in Table 1 covering a range of species including *Phaeodactylum tricornutum* [14,16], *Odontella aurita* [15], *Isochrysis* aff. *galbana* [17], *Cyclotella cryptica* [18] and *Chaetoceros calcitrans* [6]. For these studies, the maximum measured specific fucoxanthin concentration ranged between 5.25 and 18.47 mg per gram of dry algae.

Generally speaking, carotenoid pigment production is related to local environmental conditions, with light intensity and nitrogen concentration seen as the key factors [5]. Microalgae respond to changes in light levels by changing their physiology and cellular structure [5,19]. For example, when exposed to low light conditions, microalgae generally respond by increasing levels of those pigments whose primary role is light-harvesting (i.e. chlorophyll, phycobilins and primary carotenoids). Contrastingly, when the light intensity increases, the levels of these pigments often decreases, while the level of pigments which have photo-protective effects (e.g. ß-carotene) rises [5]. An example of this phenomenon is the xanthophyll cycle where the levels of photo-

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https://doi.org/10.1016/j.algal.2017.11.015
Received 4 August 2017; Received in revised form 7 November 2017; Accepted 13 November 2017

Table 1
Summary of previous work related to the production of fucoxanthin using microalgae.

Species	Cell density ($g\ L^{-1}$)	Specific fucoxanthin concentration ($mg\ g^{-1}$ dry algae)	Volumetric fucoxanthin productivity ($mg\ L^{-1}\ day^{-1}$)	Reactor type	Growth conditions	Reference
O.aurita	4–6	18.47	7.96	3 cm diameter bubble column; 75 and 150 L flat plate reactors	Modified L1 medium 100–300 μmol photons $m^{-2}\ s^{-1}$ 25 °C 1% CO_2	[15]
P.tricornutum	–	16.51	–	30 L plastic cylinders	Conway medium 2500 lx (60 W fluorescent lamps) 20 °C 5 L min^{-1} air	[14]
C.calcitrans	–	5.25	–	120 L annular reactors	Conway medium 150 μmol photons $m^{-2}\ s^{-1}$ 23–25 °C	[6,22]
P.tricornutum (six strains)	0.24–0.36	5.50	0.12	2 L flasks	f/2 medium supplemented with 0.15 mM sodium metasilicate. 40 μmol photons $m^{-2}\ s^{-1}$ 25 °C	[16]
P.tricornutum I.galbana	18.1	0.2 mg g^{-1} wet cell mass	0.20	2 L flasks with aeration or orbital agitation	f/2 and Conway media 9.1–62 μmol photons $m^{-2}\ s^{-1}$	[23]
C.cryptica	1.72	12.9	5.6	1 L flasks	10–40 μmol photons $m^{-2}\ s^{-1}$ Modified SK medium supplemented with nitrate (1 $g\ L^{-1}$) or glucose (10 $g\ L^{-1}$)	[18]

protective pigments such as zeaxanthin and diatoxanthin increase at high light intensities [12,20]. High light intensities may also induce production of astaxanthin in *Haematococcus pluvialis* where it can play a photo-protective role by absorbing blue light [21]. Overall, it is clear that the intensity of light has a large impact on the concentration of carotenoids (and other pigments) in microalgae.

Similarly, nutrient depletion (particularly nitrogen) can lead to pigment accumulation in some species [5,24]. For example, the typical industrial production processes for both ß-carotene (using *Dunaliella salina*) and astaxanthin (using *H.pluvialis*) uses two stages; in the first stage, the microalgae are grown under optimal conditions to maximise the biomass concentration while in the second stage the algae are subjected to stress (frequently a combination of nutrient depletion and increased light intensity) to maximise the carotenoid concentration [5,24].

Relatively little information about the effect of these parameters on the production of fucoxanthin by microalgae is available in the open literature, with the published studies being summarised in Table 1. Xia et al. [15] and Guo et al. [18] examined the effect of nitrogen availability and light on fucoxanthin production using *O.aurita* and *C.cryptica*, respectively. Both groups found that supplementing the media with nitrate led to a two to threefold increase in the specific fucoxanthin concencentration (i.e. from 6.71 to 18.14 mg of fucoxanthin per gram of dry cell weight for *O.aurita* and 6 to 12 mg g^{-1} for *C.cryptica*). In their work, Xia et al. [15] observed that cultures grown at a light intensity of 300 μmol photons $m^{-2}\ s^{-1}$ had a specific fucoxanthin concentration approximately two-thirds of that for cultures grown under a light intensity of 100 μmol photons $m^{-2}\ s^{-1}$; this being observed for all nitrate concentrations examined. Guo et al. [18] also found that low light intensities favoured fucoxanthin production, with a light intensity of 10 μmol photons $m^{-2}\ s^{-1}$ giving a specific fucoxanthin concentration of 10.8 mg g^{-1} while an intensity of 40 μmol photons $m^{-2}\ s^{-1}$ resulted in a specific fucoxanthin concentration of 6.2 mg g^{-1}. Gómez-Loredo et al. [23] examined the production of fucoxanthin using *I.galbana* and *P.tricornutum* finding that cultures grown using Conway medium had a greater fucoxanthin concentration than those grown using f/2 medium, however no attempt was made to evaluate the impact of supplementing specific nutrients such as nitrate.

Hence the aim of this present work is to investigate the effect of operating parameters such as the medium composition, light intensity and carbon dioxide concentration on algal growth and fucoxanthin production using *P.tricornutum*.

2. Methods

2.1. Culture conditions

In this work, we have used *Phaeodactylum tricornutum* (CS-29) supplied by the Australian National Algae Culture Collection (ANACC); the strain used was isolated in the United Kingdom; based on the available information it is most likely either accession Pt2 or Pt3 as defined by Martino et al. [25]. When examined under the microscope (Nikon ECLIPSE Ci-L) cells were observed to be predominately fusiform in nature (as opposed to oval or triradiate [25]). The algae were maintained in 100 mL flasks (50 mL medium volume) at a temperature of 25 ± 3 °C and a light intensity of approximately 30 μmol photons $m^{-2}\ s^{-1}$. Here we have used f/2 medium (without the addition of vitamins), the final composition of the medium used was $NaNO_3$ (880 μM), NaH_2PO_4 (36 μM), Na_2SiO_3 (140 μM), $FeCl_3$ (12 μM), $CuSO_4$ (41 nM), $ZnSO_4$ (76 nM), $MnSO_4$ (940 nM), Na_2MoO_4 (37 nM) and $CoCl_2$ (37 nM). To this 35 g L^{-1} of a commercially available marine salts preparation was added. This medium was used for both the maintenance of the algae as well as the growth experiments.

Experiments to quantify the effect of operating parameters on the algal growth and fucoxanthin production were performed using flat panel photo-bioreactors constructed from clear acrylic as shown schematically in Fig. 1. A total liquid volume of 5 L was used, with losses due to evaporation or sampling being replaced with deionised water such that the total liquid volume was maintained at 5 L for the duration of the experiment. Cultivations were performed at room temperature (25 ± 4 °C). Air and CO_2 were introduced at the base of the bioreactor through a 6.25 mm diameter stainless steel tube with evenly spaced 1 mm holes. The flow rates of air and CO_2 were measured using RMA series rotameters (Dwyer Instruments). Air was introduced at a rate of 5 L min^{-1}, for all experiments. Cultivations ran for a total duration of 13 days (with the day of inoculation being day zero). The 5 L photo-bioreactors were inoculated with 110 mL of algae which had been cultured in flasks for 7 days prior to inoculation; at this time the flasks had an optical density (measured at 600 nm) of approximately 0.5.

To investigate the effect of the medium composition, experiments