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Halo-adapted microalgae for fucoxanthin production: Effect of incremental increase in salinity

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ABSTRACT

In order to commercially exploit microalgae for the production of fucoxanthin, species must remain productive in the increasingly saline environment typical in outdoor cultivation ponds. To this end, this study investigated the salinity range, growth, fucoxanthin content and productivity of two halotolerant and four marine microalgae under salinity increase condition. The semi-continuous cultivation followed by gradual salinity increase and slow adaptation helped saline microalgae to extend the salinity range up to 55%. Tested species showed about 12% to 90% more fucoxanthin content at their optimal salinity compared to when grown at non-optimal salinities. Fucoxanthin productivity was found directly linked to biomass productivity. Marine microalgae performed best at salinities < 55‰ (ppt, parts per thousand) and halotolerant microalgae was best at salinities > 55‰. Among marine species, the highest fucoxanthin content and productivity was observed in *Chaetoceros muelleri* which was 2.92 mg g^{-1} and 0.072 mg L^{-1} d^{-1} of ash free dry weight (AFDW), respectively, at 45‰ and fucoxanthin content was relatively consistent over the range of salinity between 35 and 55‰. Between two halotolerants, *Amphora* sp. showed the highest content and productivity of fucoxanthin which was 1.2 mg g^{-1} and 0.053 mg L^{-1} d^{-1} of AFDW, respectively at 85‰ salinity. The results indicate that it is most likely possible to achieve continuous production of fucoxanthin by cultivating marine and halotolerant species one after another when salinity rises due to evaporation. Details of fucoxanthin content and productivity for *Chrysotila carterae*, *Chaetoceros muelleri*, *Amphora* sp. and *Navicula* sp. are reported for the first time.

1. Introduction

Saline microalgae are well known for their production of high value products such as β-carotene and omega 3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) [1]. Recently fucoxanthin, one of the predominant pigments contained in brown saline algae, has drawn public attention due to its wide range of bioactive properties. Reports have shown positive effects of fucoxanthin in inflammatory, cancer, obesity, arthritis, bone and cerebrovascular disease states [2]. A problem with more wide spread assessment and utilization of this compound is supply at a reasonable cost. Macroalgae (e.g. *Eisenia*, *Laminaria*, *Undaria*, *Sargassum*) are currently the major source of fucoxanthin [2,3], but recent studies have found that macroalgal fucoxanthin production is not economically feasible due to low fucoxanthin content (0.28–2.4 mg g^{-1} dry weight (DW) of culture) [4,5]. However, fucoxanthin is much more abundant in some saline microalgae e.g. brown saline microalgae (e.g. diatoms and haptophytes) contain about 65 times more fucoxanthin (per gram) than macroalgae (Table 3) [2,4,6–8]. On the other hand, microalgae offer advantages like high biomass productivity, high tolerance to salinity, and continuous stock supply, etc. [9]. Identification of more fucoxanthin producing microalgae can expand the opportunity of selecting suitable species from a broad range for commercial exploitation.

For commercial production of microalgal biomass and products derived from this biomass, open ponds are widely used as a sustainable cultivation system because they are easier to build and operate than other available systems [10]. To be truly sustainable the open pond systems should rely on seawater thus reducing dependence on a limited fresh water resource. If only seawater is used as a culture media in open ponds, the salinity of the media will rise due to evaporation from the pond itself. Therefore, microalgae with a wide salinity tolerance range that can sustain sufficient biomass productivity under saline conditions require for sustainable, economical production of fucoxanthin. To date, microalgae investigated as alternate sources of fucoxanthin, have been

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grown at, or below, normal seawater salinity levels (35‰) (ppt, parts per thousand) [2,3]. Such conditions are not representative of open pond cultivation where no fresh water will be added to maintain a particular salinity and other abiotic factors will be uncontrolled. Thus investigating species adapted to highly saline conditions should give a more realistic assessment of the potential of microalgae for commercial fucoxanthin production in open ponds.

The organic content of saline microalgae is known to respond to changes in salinity in natural seawater [11]. For example, artificial production of β-carotene, the most common commercially produced saline microalgal pigment, is reported to be positively influenced by culturing in increased salinity, as were other carotene and xanthophyll pigments such as zeaxanthin [12]. However, this is not a universal response for pigment production, as the same study showed that lutein production exhibited an inverse correlation to salinity increase. Although salinity is a key driver of microalgal productivity and pigment synthesis, surprisingly there are no reports on the effect of salinity on fucoxanthin production in the open literature. Therefore, it is necessary to investigate the effect of salinity on the fucoxanthin content in saline microalgae before selection of a potential strain/s for commercialization.

Given the lack of specific information regarding the effects of open pond cultivation conditions on fucoxanthin production in saline microalgae, the purpose of the study was twofold: (1) to investigate the growth characteristics of marine and halotolerant brown microalgae known to have been cultured successfully in outdoor ponds, and; (2) to investigate the effects of salinity on fucoxanthin production in those microalgae. Chlorophyll *a* and total carotenoids content, biomass productivity and maximum quantum yield of photosystem II (PSII) were also monitored to determine if a causal link existed between biomass productivity, maximum quantum yield and fucoxanthin production.

2. Materials and methods

2.1. Species selection, culture condition, media, and cultivation

Four diatoms (*Amphora* sp., *Chaetoceros muelleri*, *Navicula* sp. and *Pheodactylum tricornutum*) and two haptophytes (*Chrysotila carterae* and *Tisochrysis lutea* (T.Iso)) from two different salinity tolerance ranges (marine and halotolerant) were selected for this study. The origin of these species is summarized in Table 1. Of them, *C. carterae*, *C. muelleri*, *P. tricornutum* and *Tisochrysis lutea* (T.Iso) are marine microalgae and *Amphora* sp. and *Navicula* sp. are halotolerant microalgae. Marine or low saline microalgae typically grow at low salinity, and their salinity tolerance range usually varied from 35‰ to 50‰ (ppt, parts per thousand), and in halotolerant or mid saline microalgae, the salinity range is reported up to 77‰ [13–16]. All these species have been grown successfully in open ponds [17–20], hence their selection for the present study.

Table 1
Marine and halotolerant microalgae used in present study.

Saline microalgae		Origin details		
		Supplier	Code	Source
Marine	*Chrysotila carterae*	NCMA	CCMP647	Salton Sea, Salt Lake, CA,USA
	Chaetoceros muelleri	CSIRO	CS176	USA, North Pacific
	Pheodactylum tricornutum	CSIRO	CS-29/7	United Kingdom
	Tisochrysis lutea (T.Iso)	CSIRO	CS-177/7	Mataiva, Society Islands, Tahiti
Halotolerant	*Amphora* sp.	Murdoch University	MUR 258	Unknown
	Navicula sp.	Murdoch University	MUR 259	Unknown

All species were cultivated in 250 ml Erlenmeyer flasks containing 150 ml of culture with an initial cell density of 8–12 × 10^4 cells ml^{-1}. Natural seawater (Hillary's Beach, WA, 33‰ NaCl) was charcoal filtered before media preparation [21]. The salinity (‰ NaCl, *w/v*) of water was determined by an automatic Atago refractometer (model PAL-03S). Seawater was autoclaved and cooled before preparing F + Si media (for diatoms) [21] and F + Se media (for haptophytes) [22] by adding sterile nutrient solutions. The cultures were grown at 25 ± 3 °C under 150 μ mol photons $m^{-2} s^{-1}$ light irradiance on a 12 h: 12 h light: dark cycle. It has been found that most of the species tested here showed P_{max} (maximum photosynthetic rate) at or near 150 μ mol photons $m^{-2} s^{-1}$ [23–26]. Irradiance was measured by using a Li-185B quantum meter equipped with a PAR Li-190SB quantum sensor (LI-COR Inc., Model No. - LI-185B, Serial No. - QRPB 1443-8208, made in USA). The cultures were mixed at 100 rpm using 40 mm magnetic stirrer.

All species were grown originally at 35‰ salinity. The culture salinity was increased by 2‰ (using NaCl) at each harvest with a 72 h interval. This protocol imitated the rate of salinity increase in Geraldton, Western Australia (28.7774° S, 114.6150° E) (Fig. 2, Table S4). Geraldton was previously recognized as a potential site for large-scale microalgal biomass cultivation [27].

The cultures were maintained in semi-continuous mode and after every 72 h, a maximum 50% of the total culture volume was harvested and the same amount of fresh medium was added to the harvested flask [28]. Each time the salinity of the culture media was increased by 2‰. Each algal species was cultured up to their maximum salinity tolerance range. The harvested biomass used to measure biomass productivity, fucoxanthin content and productivity, chlorophyll *a* content, total carotenoids content and productivity, and maximum quantum yield. All experiments were carried out in four replicates.

2.2. Salinity tolerance range

The range of salinity tolerance can be determined using growth or rates of survival, photosynthesis, and respiration [29]. In this experiment, biomass productivity was used as a marker to determine the upper and lower limit of salinity tolerance range. At low salinity, the minimum biomass productivity indicated the lower limit of salinity tolerance whereas, at high salinity, it indicated the upper limit of salinity tolerance range [29].

2.3. Culture sampling

Samples were harvested using Whatman 2.5 cm GF-C filters. The harvested microalgae cultures were then rinsed with isotonic ammonium formate to remove remaining salt as previously described by Fon Sing [30]. Samples were stored at − 80 °C until further analysis.

2.4. Biomass productivity determination

The ash free dry weight (AFDW) and biomass productivity were measured using methods described in Moheimani et al. [31]. Before filtration, the GF/C filters were washed in deionized water and dried at 75 °C for 24 h and the dry weights of filters were determined. Pre-weighted filters containing 5 ml of harvested samples were then dried at 90 °C for 4 h and the total dry weight was determined by subtracting the weight of the filter from the total dry weight. Dried filters were then ashed at 450 °C for 7 h and cooled overnight in a vacuum desiccator before re-weighing. The ash-free dry weight was calculated after subtracting the ash weight from the total dry weight. Biomass productivity was measured by calculating AFDW increase over time [31].

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