



## Effect of environmental factors on $\beta$ -carotene production in green algae – A review

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Received: Jul, 21/ Revised: Aug 17, 2023/ Accepted: Aug 20, 2023

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### Abstract

Microalgae are microscopic autotrophic organisms that are typically seen in freshwater and ocean water. ecosystem and they are considered among the fastest organisms because they have a very fast life cycle Growth on Earth, as they use all available materials for growth, and they contain many effective compounds of biological, pharmacological and environmental importance. Algae contain carotenoids, which are granules that give a yellow-orange color and are found within the plastid. Carotenoids generated from microalgae are a class of accessory pigments that have the capacity to absorb light and are crucial for metabolic processes. A very small number of microalgae have been employed for the commercial production of carotenoids, although around a thousand carotenoids have been reported to date. Beta-carotene is one of the most important types of natural carotenoids synthesized by plants, and algae. In recent years, interest in it has increased greatly, and the reason for this is due to the large number of evidence indicating its benefits and importance to human health. The carotenoids of microalgae origin were briefly examined in this review. In addition, determining the effect of Salinity, light intensity, nutrients, and pH on the production and quantity of beta-carotene in some species of Green algae (Chlorophyta).

**Keywords:** Chlorophyta,  $\beta$ -carotene, some Environmental Factors.

### Introduction

The ubiquitous group of organisms known as algae can be found in a variety of settings, including water (aquatic algae), land (terrestrial algae), and even space. It is possible to say that algae are of universal occurrence because they may develop as endophytes, or epiphytes, under extreme environments. A majority of the algae are aquatic and can be found growing in freshwater lakes, pools, ponds, tanks, and rivers as well as brackish water (which should not be drunk) and marine water (with moderate to extremely high salinity). (Baweja and Sahoo, 2015).

### Green algae (Chlorophyta)

The single-cell or multicellular colony-developing species are covering the plant's sphere. They contain carotenoids such as carotene, xanthophylls, lutein, and pyrenoids as well as chlorophylls (A and B). They appear green because chlorophyll is found in green. In some cases, polysaccharides with cellulose as an ingredient make up the cell wall. They

occasionally produce a "thallus" by displaying only partial variants. Complex cell differentiation does not exist in multi-cellular cells. Carbohydrates are stored as oils and starches in the photosynthesis product. Over 9,000 species have been identified, 10% are in seas, while 90% are freshwater. They are widespread throughout the early spring, late summer, and autumn seasons. They contribute to the formation of lichen. (Baweja and Sahoo, 2015).

### *Dunaliella salina*

One of the most important green algae known to produce beta-carotene is the genus *Dunaliella* They are unicellular, ovoid, lacking a rigid cell wall (naked), containing It has a single large green stem in the shape of a cup and are moved by two long, equal flagella. The length and appearance of it are similar to *Chlamydomonas* and the main difference between them, It is the absence of a cell wall in *Dunaliella* algae (Borowitzka and Borowitzka, 1992 and Gonzalez et al., 2005). Due to its high concentrations of beta-carotene inside its cells and the potential for use as an industrial source for the

synthesis of beta-carotene (Massyuk and Abdula, 1969; Massyuk, 1966). Many researchers working in the pharmaceutical, cosmetic, and nutraceutical industries are becoming increasingly interested in *Dunaliella* (Marino et al., 2020; Roy et al., 2021).

The species *Dunaliella* is regarded as an important supplier of vitamins, lipids, proteins, and carbohydrates. (Hosseinzadeh Gharajeh et al., 2020). As a result, it provides *Artemia* with its main natural source of nutrition and is an excellent feed for fish and crustaceans, such as shrimp, prawns, and fish larvae. (Mohebbi et al., 2016). On the other hand, *D. Salina* enhances *Artemia sp.*'s nutritional status, according to Hannah et al. (2013). In the aquaculture sector,  $\beta$ -carotene, a pro-vitamin A source is utilized as a natural feed addition to enhance immune function and disease resistance since it has antioxidant properties (Madhumathi and Rengasamy, 2011). Due to the accumulation of  $\beta$ -carotene and its capacity to convert into astaxanthin, *In addition to promoting growth, D. salina improves P. monodon's immunity, general health, and ability to resist disease.* Additionally, *D. salina* meal supplementation promotes white shrimp *Penaeus indicus* growth and body color to achieve the desired color of the skin and flesh (Ahmad et al., 2015). Due to the difficulty of identifying *Dunaliella* only by morphological-based taxonomic analysis, numerous taxa have been incorrectly recognized in published works of literature due to the absence of a hard cell wall, which in turn results in great phenotypic flexibility (Oren, 2005; Borowitzka and Siva, 2007). Therefore, to accurately identify species and even distinct strains of *D. salina*, physiological, biochemical, and molecular features were used (Olmos et al., 2000; Olmos-Soto et al., 2002; Hejazi et al., 2010).

### Carotenoids

One of the most intriguing, plentiful, and common classes of natural pigments is carotenoids. Carotenoids are produced by a variety of photosynthetic organisms, a variety of non-photosynthetic bacteria and fungi, as well as oxygenic photosynthetic bacteria, cyanobacteria, algae, and higher plants (Goodwin, 1980).

The term "carotene" was initially put forth in 1831 by Wackenroder to refer to the pigment he had extracted and crystallized from the roots of carrots. Berzelius created the name "xanthophyll" in 1837 to chemically describe a yellow pigment that he had chemically isolated from senescent leaves.

Today, the term " $\beta$ -carotene" is used to describe a hydrocarbon carotenoid, possibly a derivative of carotene that has one or more functional groups that include oxygen are referred to as "xanthophyll".

Bacteria, microalgae, cyanobacteria, plants, and fungi all contain carotenoids, which are predominantly lutein and  $\alpha$ -carotene, two naturally occurring yellow to orange-red chemical compounds with a polyisoprene backbone. (Concepcion et al., 2018; Kanzy et al., 2015; Saini and Keum, 2017). Reactive oxygen species (ROS), a well-known class of

molecules that can lead to oxidative stress, can be removed by carotenoids (Britton et al., 2008).

### Chemical Structures of Carotenoids

According to their structural characteristics, carotenoids are divided into two classes: "hydrocarbon carotenes" and "xanthophyll" (Armstrong and Hearst, 1996).  $\beta$ -Carotene,  $\alpha$ -carotene,  $\gamma$ -carotene, lycopene, phytofluene, and phytoene are some of the hydrocarbon-carotene's polar components. Zeaxanthin, lutein, fucoxanthin,  $\beta$ -cryptoxanthin, and astaxanthin are a few xanthophyll examples. The oxygen in the form of (hydroxyl, methoxy, epoxy, carboxy, and keto) is found in the more polar xanthophyll (Eldahshan and Singab, 2013; Jaswir et al., 2011).

There have been reports of numerous carotenoids in various microalgae, including lutein, zeaxanthin, astaxanthin, canthaxanthin, and fucoxanthin. (D'Alessandro et al., 2016). Particularly, (Table 1) describes the chemical structures of carotenoids.

The process of producing carotenes can be visualized as an upside-down tree, with the trunk standing for the initial reactions that all living things experience and the branches signifying the enormous variety of xanthophylls produced by the species-specific addition of oxygen functionalities to acyclic and cyclic carotenes (Fig. 1). Thus, these oxygen functions, represented by carboxy, hydroxyl, epoxy, oxo, methoxy, and aldehydic groups, serve as the primary foundation for the structural diversity of the carotenoids. (Goodwin, 1980; Straub, 1987). Emeish, (2013) showed that there are typically 4 stages in the biosynthesis of " $\beta$ -Carotene".

1. Mevalonic acid is a source of (Geranylgeranylpyrophosphate) GGDP.
2. Phytoene is formed by condensation.
3. Phytoene desaturation to lycopene.
4.  $\beta$ -Carotene is created when lycopene hydrolyzes.

A highly important carotenoid pigment known as  $\beta$ -carotene has a wide range of applications in the food, cosmetics, and pharmaceutical industries (Coppens et al., 2016; Gateau et al., 2017; Paillie-Jimenez et al., 2020). Carotenoids have health benefits in addition to serving as colorants and are effective in avoiding cataracts, macular degeneration, and cancer. Carotenoids have health benefits in addition to serving as colorants and are effective in avoiding cataracts, macular degeneration, and cancer (Mussagy et al., 2019). Given that  $\beta$ -carotene has considerable effects on animal pigmentation and high antioxidant activity, the supply of natural  $\beta$ -carotene now falls short of the demand (Henriquez et al., 2016).

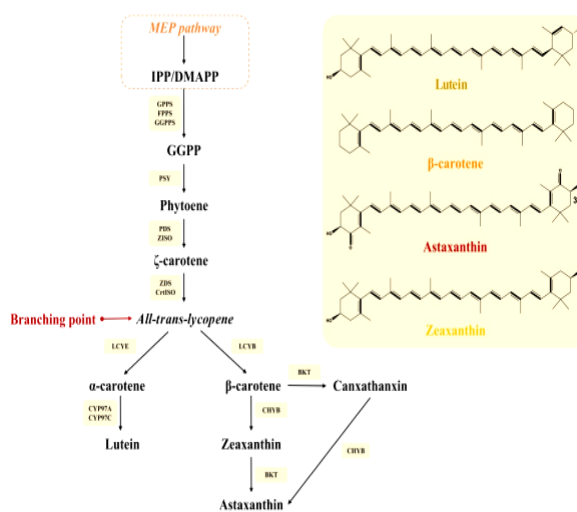
*Dunaliella* may respond to stress conditions like high salt concentration, high light intensity, nutrient stress, and pH extremes by producing and accumulating enormous commercially and economically significant amounts

significant chemicals, such as  $\beta$ -carotene and glycerol (Hadi et al., 2008; Lin et al., 2013). Additionally, according to (Ben-Amotz 2007), Environmental conditions played a major role in determining the amount of carotenoid *Dunaliella spp* produced. High temperatures, high light intensities and High

salinity are associated with the greatest quantity of beta-carotene production. For the purpose of producing a lot of carotene and glycerol, Different *Dunaliella sp.* were cultivated (Phadwal and Singh, 2003).

**Table 1.** lists the chemical composition of some common micro algal carotenoids

| Carotenoid        | Chemical Structure |
|-------------------|--------------------|
| Astaxanthin       |                    |
| $\beta$ -carotene |                    |
| Lutein            |                    |
| Zeaxanthin        |                    |
| Canthaxanthin     |                    |
| Violaxanthin      |                    |
| Lycopene          |                    |



**Fig. 1** Shows the chemical structures and biosynthesis processes of carotenoids in microalgae

## The benefits of Carotenoids

Carotenoids are a group of terpenoid pigments having C40 backbones that are beneficial to human health when consumed often. Over 1100 carotenoids have been identified so far, and they can be found in a variety of animals (Liu et al., 2021), particularly in microorganisms, terrestrial plants, and aquatic creatures. The presence of carotenoids is ubiquitous in our daily lives. For instance, lycopene (a pink-red pigment) is abundant in tomato fruit and maize corn is a rich source of zeaxanthin (yellow), and beta-carotene (orange) is abundant in carrots and *Dunaliella salina* (Liu et al., 2021). Humans who consume carotenoids may use them as precursors to vitamin A (Qiang et al., 2019), reduce free radicals (Qiang et al., 2019), and repair damaged retinas (Roberts et al., 2009). Additionally, because they have anti-inflammatory, anti-obesity and anti-cancer effects, they can help lower the risks of various diseases (Le Goff et al., 2019). As a result, the food, cosmetic, feed, and pharmaceutical industries have extensively exploited carotenoids.

## Impact of Environment Factors on $\beta$ -Carotene Production

Numerous environmental elements, including salinity, pH, light, temperature, and nutrients, have an impact on the amount of  $\beta$ -Carotene that algae produce. So, research on physically controllable parameters (cell density, culture, dilution cycles and depth) that affect productivity is required to determine the performance of a prototypical pilot plant for the production of *Dunaliella* in the considered area.

### Salinity

The high cell content of  $\beta$ -carotene, protein, or glycerol and the capacity to grow at very high salt concentrations make this microalga an appealing choice for the commercial production of these substances (Spolaore et al., 2006; Hosseini-Tafreshi and Shariatie, 2009). The results of numerous studies on the effects of salt stress on *D. bardawil* show that cell count, chlorophyll a, and beta-carotene contents increased gradually and significantly as sodium chloride concentration increased, reaching their maximum values at 2.5 M, before gradually declining to their minimum values at 4M NaCl. According to the results published by Rad et al. (2011), salt stress had an impact on both overall carotenoid production and cell productivity, and *Dunaliella sp.* treated with 2 M NaCl produced the highest levels of carotenes per cell while Loeblich (1982) discovered that *D. salina* cells' content of  $\beta$ -carotene increased as salinity increased.

### light intensity

One common stress that microalgae may encounter in certain environmental circumstances is exposure to intense light. In fact, during the middle of the day, the light irradiance may approach 1800 mol photons  $m^{-2} s^{-1}$  (Faraloni and Torzillo, 2017). Antioxidant carotenoids' synthesis is stimulated in response to this buildup of extra energy, which causes ROS to

formation, protecting the cells from photodamage. It is believed that the findings of (Ben Amotz and Avron, 1983) about the higher ratio of  $\beta$ -carotene to chlorophyll at high irradiation indicate that  $\beta$ -carotene has a protective effect against injury caused by high irradiance. The results of their investigation are supported by their demonstration that  $\beta$ -carotene accumulation was associated with chlorophyll depletion at high light intensities under conditions of impaired chlorophyll content per cell. According to (Telfer, 2002) beta-carotene acts as a light filter, quenches chlorophyll in its triplet state, inhibits the creation of reactive oxygen species, and shields algae from damage brought on by excessive irradiances.

Different metabolic pathways may be followed by the induction mechanism based on the type of light and the strain. For instance, the xanthophyll cycle, which is known to occur very quickly, maybe induced within 15 to 30 minutes of the cells being abruptly exposed to high light intensity (Niyogi et al., 1997).

The red pigment astaxanthin is one of the most significant secondary carotenoids created by microalgae. *Haematococcus pluvialis* produces this extremely potent antioxidant, particularly when exposed to strong light. The green cells must be exposed to sunlight for one day before they change red, therefore even if its synthesis is slower, it can still reach quite high content; it accounts for 5% of the biomass. Due to the high astaxanthin production of this carotenoid and its robustness, *H. pluvialis* has been the subject of extensive research. In reality, the majority of studies on *H. pluvialis* have been carried out in outdoor cultures where astaxanthin production is stimulated by sunshine. This research shows that this microalga may flourish and produce astaxanthin under specific environmental circumstances, particularly during the summer and in locations with extremely high illumination (Torzillo et al., 2003; Recht et al., 2014).

### Nutrients

Since nitrogen is one of the most vital components in the cell and is found in proteins, nitrogen deficiency is one of the diseases associated with nutritional stress that has been studied the most (Faraloni and Torzillo, 2017).

Carbon and nitrogen are important macronutrients for microalgae growth and flourish. The carbon source can be created synthetically by incorporating carbon-containing substrates like glycerol, urea, glucose, etc., or it can originate from the atmosphere (CO<sub>2</sub>) (Bhatnagar et al., 2011). Numerous studies have been conducted in recent years to determine the best conditions for microalgae to produce  $\beta$ -carotene. For instance, it has been discovered that high temperatures, high irradiance, oxidative stress, and nitrogen deprivation all greatly promote  $\beta$ -carotene accumulating in microalgae (Fachet et al., 2016; Wu et al., 2016).

The production of carotenoids is influenced by environmental factors such as nutrients, and as with high light irradiance, a



deficiency of macronutrients causes more severe protective responses than a deficiency of micronutrients. It has a direct effect on growth and is principally responsible for increasing the reducing power when combined with light exposure, which is known to activate defense mechanisms such as promoting the synthesis of certain carotenoid receptors (Faraloni and Torzillo, 2017). Nitrogen limitation is one of the nutrient-deprivation stresses that has been the subject of the most research due to its significance in the cell, its presence in proteins and enzymes, and the direct role it plays in growth. Carotenoid levels increase in this microalga, just as was previously shown in *Dunaliella* under severe light stress, and this also happens in a nitrogen starvation state. It has been demonstrated, for example, that the synthesis of total fatty acids occurs concurrently with an increase in beta-carotene content when there is a nitrogen deficiency and excessive sun exposure. This is supported by the observation that lipid globules and  $\beta$ -carotene cannot be detected in the presence of fatty acid biosynthesis pathway inhibitors, which is explained by the fact that  $\beta$ -carotene accumulates in lipid globules within cells (Rabbani et al., 1998). When nitrogen is low, *D. salina* can achieve 2.7% of its biomass as  $\beta$ -carotene at light intensities of 200 mol photons  $m^{-2} s^{-1}$  (Lamers et al., 2012).

In *H. pluvialis*, the relationship between carotenoid synthesis and lipid carotenoid synthesis has been investigated. In particular, high light and nitrogen starvation together have been shown to cause the greatest accumulation of carotenoids; under these circumstances, the astaxanthin content was more than twice as high as the control (Liang et al., 2015).

Astaxanthin synthesis is increased when nitrogen is starvation compared to the control culture. Astaxanthin synthesis happens during the change from the green to the red stage due to the cytoplasmatic accumulation of the red pigment, which is detected after 20 hours and reaches 1.4% of dry weight in the starved culture.

A growth medium with different carbon and nitrogen ratios can also increase the capacity of microalgal cells to produce carotenoids. (Contreras et al., 2020).

It has been noted that *Chlorella zofingiensis* astaxanthin concentration increases with the addition of carbon substrate to the medium. Astaxanthin production occurs in the absence of light. is accelerated by the addition of carbon substrates, including malate, pyruvate, and citrate (Bhatnagar et al., 2011).

In the synthesis of nucleic acids, proteinaceous substances, and pigment molecules, nitrogen is an essential macronutrient. in addition to having a substantial impact on how microalgae regulate their metabolism. Microalgal cells are stressed by the absence of nitrogen in the media, which could increase the synthesis of carotenoids (Guedes et al., 2011).

Under nitrogen-limited conditions, *Dunaliella salina* showed an increase in  $\beta$ -carotene, according to Pisal and Lele's study (Pisal et al., 2005). When nitrogen was starved, the content of  $\beta$ -carotene increased from 1.65 pg/cell to 7.05 pg/cell. However, a number of study authors noted that nitrogen limitation can cause oxidative stress. Nitrogen starvation caused oxidative stress in *Chlorella sorokiniana*, according to a study by (Zhang et al., 2013).

## pH

The hydrogen ion concentration (pH) of the culture medium has a significant impact on the best possible growth of algal cultures. It seemed that different algae species have distinct optimal pH for growth. Marine *D. tertiolecta* growth requires a pH of approximately 6 (Craigie and McLachlan, 1964). Whereas *D. salina* and *D. viridis*, which are halophilic, require an optimum pH of about 9. The optimum pH for photosynthesis is often lower than the optimum pH for growth, according to research by (Loeblich, 1972; Wegmann and Metzner, 1971). The algal culture density and starting cell concentration should be considered in the design and volume of culture systems. *Dunaliella spp.* were grown in non-axenic outdoor mass cultures for two to three days in f/2 and 2f media, and it was discovered that the final concentration was based on the initial cell density, as cultures with  $80 \times 10^3$  cells per milliliter produced better yields than those with  $40 \times 10^3$  cells per milliliter (Becerra Dorame et al., 2010). Optimizing the algal density at which the greatest biomass and carotenoid content are accomplished in ponds and photobioreactors is an essential step (Hosseini-Tafreshi and Shariatie, 2006).

The control culture of *D. bardawil* required a pH of 7.5 in order to grow and produce glycerol. The values of these parameters were dramatically reduced when the pH was shifted to either the acidic or alkaline side. However, *Dunaliella* can grow in a wide pH range (5.5-9.5), as shown in the literature. This intrigued organism can therefore withstand a wide range of pH, which may add to its advantages. These results supported those of Essa (1995), who discovered that *Dunaliella sp.* had a maximum cell count at a pH of 7.8 and that a pH change to 6 or 9 had a minimal effect on the number of *Dunaliella* cells. He also mentioned that 7.8 was the optimum pH for the synthesis of glycerol in *D. salina* and *D. parva* cells. The research also demonstrates that the value of  $\beta$ -carotene reduced considerably at both lower and higher pH levels, with pH 8.5 being the optimal value. The results of Anon (1983) and Khalil et al. (2010), who noticed that pH 9.0 produced the greatest value of  $\beta$ -carotene in *D. bardawil*, are suitable for this. On the other hand, Muthukannan et al. (2010) discovered that *D. salina* needs an alkaline pH of (7.5) in order to produce most of the important  $\beta$ -carotene pigment.

## Conclusions

The major source of useful pigment molecules, such as carotenoids, which have coloring and antioxidant properties, is microalgae. According to the environmental factors,

various microalgae produce varied amounts of carotenoids including vialoxanthin, lutein, astaxanthin,  $\alpha$  and  $\beta$ -carotene, zeaxanthin, fucoxanthin, and neoxanthin.

One of the best sources of natural carotene is *Dunaliella Salina* which collects a high content of carotenoids up to (10%) from Dry biomass under suboptimal growth conditions such as high light intensity, Sub-optimal temperature, nutrient deficiencies, and high salt concentrations. It has been proven that *D. Salina* algae can produce and accumulate high concentrations of beta-carotene (more than 15% of dry weight) in oily granules in the cell under the influence of certain environmental factors such as a lack of nutrients, excessive salinity, and intense light. Therefore, Carotenoids are affected by many environmental factors, such as some nutrients (phosphorous & nitrogen) and high salinity, and pH, as beta-carotene accumulates among the thylakoids in the chloroplast that form vitamin A.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

### Funding support

The author declares that they have no funding support for this study.

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**How to cite this article**

Al-Khafaji, A. S. and Al-Taei, I. A. (2023). Effect of environmental factors on  $\beta$ -carotene production in green algae – A review. *Chemical and Environmental Science Archives*, Vol. 3(3), 48-54. <https://doi.org/10.47587/CESA.2023.3301>

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