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POTENTIAL ISOLATES OF *Dunaliella salina* FOR THE NATURAL BETA-CAROTENE PRODUCTION: A REVIEW

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ABSTRACT

Dunaliella salina a halo tolerant microalgae. For the production of natural beta-carotene at commercial level is widely studied nowadays. ***Dunaliella salina*** is one of the largest source of beta-carotene (i.e. 14% of dry weight of cell). Basically ***Dunaliella salina*** is the green microalgae but when there is an extreme conditions it accumulate the beta-carotene for protection. And hence it appears orange in colour due to accumulation of beta-carotene.

Objectives:

The main objective of this review is to compare the growth and accumulation of beta-carotene under different stress conditions which give scientific guidance for commercial algal production.

Keywords: *Dunaliella, salina,* tocopherols, beta-carotene, stressful conditions, photoinihibition, photobioreactor, Johnson media

INTRODUCTION

Microalgae are fastest growing autotrophs on the earth. Microalgae are photosynthetic microorganisms. Some microalgae is used for human consumption as a source of high value health food, functional foods, and for production of biochemical products, such as vitamins, carotenoids, phycocyanin and polyunsaturated fatty acids including the omega-3 fatty acids have been developed **[1]**. Microalgae are rich source of bioactive chemicals which has health benefits like cholesterol reduction, anti-arthersclerostic or anti-cancer properties.

The unicellular alga *Dunaliella salina* is known as most salt tolerant algae. *D. salina* has also achieved prominence as a commercial source of 'natural beta- carotene for food and feed industry. This is because this alga provides the richest natural source of beta-carotene, with beta-carotene contents of > 10% of dry weight. *Dunaliella salina* was first recognized by Teodoresco (1905). This alga is mostly found in natural marine habitat, due to which the water appears red in color. *Dunaliella* contains high concentration of beta-carotene [a]. Beta-carotene is orange pigment which is lipid soluble and antioxidant, which is synthesized by high plants and microorganisms, which is used in cosmetics and as a colorant for feed and food [b]. It is a important source of nutrition, because it can be converted into vitamin A. In recent studies, as a food supplement D. Salina, having high beta-carotene level is a safe food supplement. Beta-carotene nowadays has many application and used as a colorant, a food additive an antioxidant, an anticancer agent, and in cosmetic purpose **[2]**. Beta-carotene is produced chemically, but there is a considerable interest in production of natural beta-carotene from living organisms **[2]**. Among the living organism producing beta-carotene naturally, *D. Salina* is a unicellular green alga belonging to chlorophyceae family. *Dunaliella* cells are found in different shapes like ovoid, spherical, pyriform, fusiform, or ellipsoid with size varying from to 25 micrometer in length and from 3 to 13 micrometer in width. Multiplication of Dunaliella occurs by lengthwise division, but sexual reproduction does occur rarely by isogamy with a conjugation process. The intracellular glycerol content exceeds 50%, when dunaliella is grown at high salinity. In such conditions, glycerol acts as a 'compatible solute', which protects enzymes against both inactivation and inhibition **[3]**. These organisms can able to adapt the most of the sever conditions of the global habitats. Some examples of these organisms are: dunaliella acidophila can grow in the pH of (0-1) which acidic condition. Dunaliella Antarctica survive at subzero temperatures and some organisms tolerate the high light intensities. *D.salina* accumulates the beta-carotene under various stress conditions, such as high salinity, high light intensity and low growth temperature, difference pH, etc **[2]**. Under these stress condition, the normal physiological balance of the cell gets disturb such as formation of free radicals. For protection and continuous growth, the cell accumulates the beta-carotene. D. Salina, D. bardawil, D. parva, D. tetrilecta, and D.

viridis have been reported commercially interested. Main morphological attributes are naked cells, flagella and a pyrenoid, chloroplast in cup form rich in chlorophyll a & b, xanthophylls & carotenes (alpha, beta & gamma) **[3]**. It is also well known for its high content in lipids, which includes polar lipids, which are useful for nutraceutical and cosmetic applications. At the industrial level *Dunaliella salina* is produce as dried algal cells. It is used as a food supplement and it is known for its antioxidant properties, due to high carotenoids contents. It has been reported by some authors that *D. Salina* can accumulate the large amount of carotenoids, alpha-tocopherol and ascorbic acid, which enhanced the activities of antioxidant enzymes when they are exposed to the high light intensity, and the media containing high salt concentration and or limiting nitrogen **[1]**. These antioxidant products like carotenoids, vitamin E, vitamin C. These are mainly used as health food market for direct consumption by a human which is safe **[1]**. Based on epidemiology and oncological studies suggest that humans fed on a diet high in carotenoid rich vegetables and fruits, which maintain higher than average levels of serum carotenoids, have a lower incidence of several types of cancer and degenerative diseases **[4]**. More than 10% of algal dry weight can be beta-carotene under the given stress conditions which mainly composed of two stereoisomer: all trans and 9-cis **[4]**. When the dunaliella synthesis or eliminate the glycerol to an intracellular concentration, which osmotically balance the external salinity permits the cells to resume the growth **[5]**. There are two metabolic pathways which may be responsible for the glycerol formation:

(1) Using the photosynthetic product.

(2) From the degradation of starch, storage product of Dunaliella.

In pathway of glycerol synthesis the important enzyme is G3pdh. Glycerol is chemical substance with different applications in food, chemical and pharmaceutical industries, also used as a raw material for new chemical and biochemical process **[5]**. Glycerol content declined 52.05% when salinity was changed from 2.0 to 0.5 M NaCl, and glycerol content increased 43.61% when salinity was increased from 2.0 to 5.0 M NaCl. The experiment has performed, to investigate the effect of salinity changes on the growth of dunaliella, the role of glycerol and the relationship between salinity and activity expression of G3pdh isoenzyme **[5]**.

Table: 1 different concentration of carotene in microalgae

Strain	Carotenoid	Carotenoid content	Reference
Dunaliella salina	**Beta-carotene**	**~10%**	**10**
Haematococcus pluviallis	**Astaxanthin**	**~7.7%**	**67**
Muriellopsis sp.	**Leutin**	**~0.8%**	**68**
Scenedesmus almeriensis	**Leutin**	**~0.6%**	**69**
***Dunaliella salina* mutant**	**Zeaxanthin**	**~0.6%**	**55**
***Coelastrella striolata* variant**	**Canthaxanthin**	**~4.8%**	**70**

Cellular localization and function of beta-carotene in *D.salina*

When *Dunaliella salina* exposed to the stress conditions such as high light intensity, high salinity, extreme temperatures and /or nutrient deprivation, *Dunaliella salina* can accumulate large amount of beta-carotene (~ 10% of the dry algal biomass) and due to this microalgae appears orange in color **[6]**.

Cellular localization

D. bardawil, accumulate beta-carotene in oil-containing globules, which are located in the inter thylakoid space of the chloroplast **[6]**. It is said that most of the beta-carotene containing globules are localized/ present near to the area of the plasma membrane **[6]**.

Function of beta-carotene

The function of this accumulated beta-carotene is to protect the cell from the photo inhibition which is mediated by the high light intensity **[6]**.

Carotenoid induction

It is said that accumulation of beta-carotene can protect the cell against the effect of high intensity of light. Several experiment has been done to maximize the production of beta-carotene. These experiments were done by giving them the stress conditions such as high salinities, low nutrient levels and high temperature with high light intensity which reduce the growth rate and at the same time, induce beta-carotene production in cell.

When the stress intensity is higher the cell growth rate will be slower and the light which is absorbed by the algal cell will be greater, and this leads to higher accumulation of beta-carotene per cell **[7] (Figure 1)**.

Regulation of beta-carotene accumulation

By many studies, it has been concluded that under different stress conditions *D. salina* accumulate the beta-carotene. The regulation of this beta-carotene accumulation is not completely known. But here we have little overview on the regulatory mechanism of this beta-carotene accumulation in *D. salina* and by this we can improve our knowledge in this area. There are different steps to clearly understand the regulation of carotenoid accumulation:

1. Sensing of the induction signal
2. Signal transduction
3. Regulation of carotenoid biosynthesis

Regulatory mechanism for the induction of beta-carotene overproduction (Figure 2).

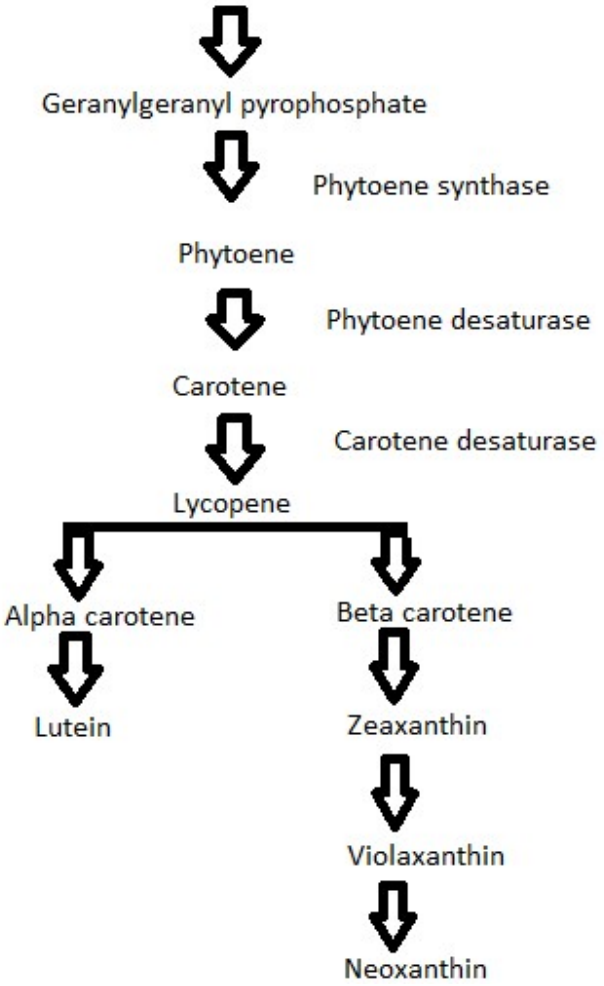

Figure 1: Pathway of carotenoid biosynthesis in microalgae

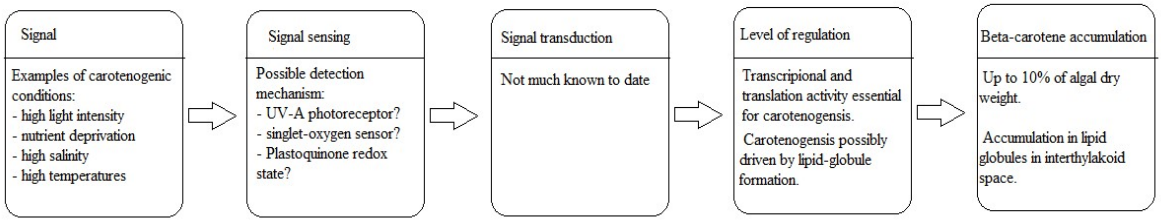

Figure 2: Regulatory mechanism for the induction of beta-carotene overproduction

Signal sensing and signal transduction

When *D. salina* cells are exposed to the stress environment, they have ability to detect the relevant changes in the environment, the key induction parameter for the beta-carotene overproduction in *D. salina* is the light intensity and there is the existence of the photoreceptor that would function as the signal sensing mechanism **[6]**. There are different mechanism which detect the signal:

- UV- A photoreceptor
- Singlet – oxygen sensor
- Plastoquinone redox state

When the photoinhibition occurs due to the UV-A, there will be the high beta-carotene production to protect the cell. It is also said that, the photo inhibition which is mediated by the UV-A is due to the effect of ROS that have been generated by the UV-A light **[6]**. Another secondary messenger, which initiate the cascade of the signal transduction, is the singlet oxygen. But a single-oxygen sensor which is able to mediate the beta-carotene accumulation is not found in *D.salina* or any algal species **[6]**.

Level of regulation of carotenoid synthesis

The carotenoid synthesisis regulated at the level of the transcription, translation or enzyme activity. The induced accumulation of beta-carotene is regulated by adding transcriptional and translational inhibitors to cultures of *D. bardawil* and then it is exposed to the high light intensities **[6]**.

Strain selection, improvement and laboratory maintenance

When we do the mass culture of *Dunaliella salina* either in open ponds or bioreactors, the prior step is to select the appropriate strain in terms of beta- carotene content and rate of growth. Among the various strains of *Dunaliella salina* can accumulate high amount of beta-carotene (i.e up to 10-14 % of dry weight) **[8]**. *D.salina* and *D. bardawil* which are able to produce large amounts of beta-carotene more than 10% of dry weight. From various marine habitats such as inland salt lakes, salt marshes and artificial salt ponds, which contains 10% of salt, the strains of *D. salina* have been isolated.

After the selection of the strain, to have the maximum specific growth rate and the highest beta-carotene under optimized conditions, we need to improve the strain of *D.sailna*, which can accumulate the good amount of beta-carotene. For improvement of the strain many scientist worked genetic diversity *Dunaliella salina*. To induce the mutation in strain of *dunaliella*, the chemical mutagen and UV are used. Recently **[9]**, has isolated and describe a spontaneous mutant, which has very short flagella. Another recent, a novel mutant was isolated by ethyl methyl sulphonate mutagenesis treatment and isolate is named as ‘ zeal **[10]**.

After selection and improvement of strain for mass cultivation of *D. salina*, the most important thing is to maintain the strains in proper culture medium and for proper growth of D. salina the culture medium is required, which provides the proper growth of *D. salina* and maintain the strain of *D. salina*.

The most commonly used media is the modifies Johnson medium **[11]**. Other media like modified ASP medium **[12]**, parvasoli-enriched seawater medium **[13]**, f/2 guillar's seawater medium **[14]** and artificial medium ART **[15]**, also been used as culture media. **[16]**, said that dunaliella alga is successfully maintained in liquid nitrogen, for up to 12 months.

Applications

1. To produce the vitamin A, the beta-carotene is oxidized by liver enzymes.
2. Beta-carotene is also used as a animal and human dietary supplements.
3. Beta-carotene is also used in food, cosmetic and pharmaceutical products as colourant / antioxidant **[17, 18]**.
4. Beta-carotene also has ability to inhibit or prevent different types of tumor in both human and animal, including skin cancer.
5. Beta-carotene is also used in controlling cholesterol level and also reduce the risk of cardiovascular diseases.
6. As we know natural beta-carotene composed of 9-cis mainly which is balance by all-trans isomer. It is proved that 9-cis isomer is known as better antioxidant where as 9 cis to all-trans ratio, is higher in antioxidant and anticancer activities.

Advantages

Dunaliella has the following advantages:

1. Due to its wall-less nature the cell disruption is very easy.
2. The growth rate is high and continous culture in laboratory is easy.
3. Compare to other algae, resistance to various environmental conditions is higher.
4. *D. salina* can produce and accumulate of carotenoids in oil globules can be enhanced is the main advantage of *D. salina*.

Stress parameters

As we know that the beta-carotene will accumulate when we exposed them to the different stress conditions. So many scientists have performed experiment to investigate the accumulation of beta-carotene under different stress conditions.

For performing the experiments under stress condition, the proper samples were collected from the natural marine habitat, the sample were collected in the previously clean and sterilized glass jars. After collection the sample of dunaliella is added in to the most commonly used Johnson media. Once the samples are collected the isolation of dunaliella has to be done.

Different stress parameters are given for the accumulation of beta-carotene:

One of the most important stress parameter is the high light intensities. When their will be the high light intensities the D. salina over produced the beta-carotene for the protection

against the photo inhibition, so it is said that light intensity is one of the most important factor that control the beta-carotene accumulation in *D.salina*. [19].

Next to the light intensity the nitrogen deficiency is one of the most important environmental factor inducing the beta-carotene accumulation in *D. salina* [20]. The accumulation of beta-carotene contents simultaneously increased in *D. salina* cells upon either high-light treatment alone or high light treatment in combination with the nitrogen depletion. [20].

One more parameter is the pH which is again an important for algal growth as it can affect the activity of different enzymes. In general different algal species have diverse ranges of tolerance to pH. *Dunaliella* species has a wide range of pH tolerance but for its optimum growth and biopigment content pH 8 was found optimum.

When there is a macronutrient deficit conditions, there will be the oxidative stress, and it is reduced by giving bicarbonate supplementation which also enhances growth and biochemical composition of *Dunaliella salina*. Maximum accumulation of carotenoids including beta-carotene was observed on addition of bicarbonate during nitrate deficiency compared to phosphate and sulphate deficiency **[21]**.

So we can accumulate the beta-carotene by giving such type of stress conditions to *Dunaliella salina*.

CONCLUSION

This review article mainly focus on potential on *Dunaliella sailna* of accumulating the beta-carotene under different stress conditions like high light intensities, salinity and high temperatures, pH, bicarbonate supplementation, nitrogen deficiency, nutrient depreivation, etc should be the stress conditions. In this review the attention is also given on the regulation of brta-carotene accumulation, induction of beta-carotene and also the cellular localization of beta-carotene with its functions. The strain selection, improvement of strain by inducing mutation in strains and how to maintain the culture is also mentioned in this review. Due to different potentialities of *Dunaliella salina*, it can be the main topic for future microalgal investigation or for more information. From this review it is concluded that *Dunaliella salina* is the best natural source of beta-carotene.

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