Proceedings of the World Congress on New Technologies (NewTech 2015)
Barcelona, Spain – July 15-17, 2015
Paper No. 149

Evaluation of an Indigenous *Dunaliella* Strain for β-Caroten and Neutral Lipid Production as a Response to Cobalt Deprivation

Zeynep Elibol Çakmak
Kırıkkale University, Department of Biology
Yahsihan, Kırıkkale, Turkey
zelibolcakmak@gmail.com

Turgay Çakmak
Istanbul Medeniyet University, Department of Molecular Biology and Genetics
İstanbul, Turkey
turgay.cakmak@medeniyet.edu.tr

Abstract **–** Microalgae offer a wide range of applications in biotechnology. Some microalgae have considerable potential to be utilized for high value added product production. Genus *Dunaliella* offer strains having considerable potential to be employed for production of high value added products such as β-caroten and triacylglycerols. In this study, an indigenous *Dunaliella tertiolecta* IMCC-37 strain was employed for its potential to be used for triacylglycerol and β-caroten production. Based on literature info, *D.tertiolecta* IMCC-37 strain was grown in Modified Jhonsons medium as controlled or Cobalt deprived conditions for one month of incubation period on a shaker with a 120rpm speed under 28^{o}C temperature and 150 PAR continuous light. Long term Cobalt deprivation did not cause considerable change in growth while photosynthesis activity increased as supported by increase in Chlorophyll-a and Chl-a/b ratio, triacylglycerol levels increased over 27% as supported by 11% increase in neutral lipid content and cells accumulated carbohydrate over 13% starting from 15th day of Co deprivation. Even if there was no significant change in total carotenoid production, β-caroten production increased over 14% at the end of 15 days of incubation period. Results show that Cobalt deprivation may be used as a potential tool for increasing both triacylglycerol and β-caroten production by *D.tertiolecta*.

Keywords**:** Dunaliella tertiolecta, Lake Meke, Cobalt, halotolerant microalgae

1. Introduction

Microalgae are single-celled, ubiquitous, primary photosynthetic microorganisms. They represent a wide array of species, inhabiting environments from deserts to the Arctic Ocean, including both salt and fresh water (Griffiths et al., 2011). Being a fundamental step of the nutrient chain, microalgae offer a wide range of metabolites which are of biotechnological importance. Regardless of their usage in agriculture and food industry as fertilizer or food supplement, they are most pronounced for production of several primary metabolite (such as protein, lipid, carbohydrate) or secondary metabolite (such as carotenoids, alcoloids), bioremediation (waste-water remediation; heavy metal absorbtion/adsorption), and biofuel (biohydrogen, biodiesel etc.) production (Lewis et al., 2000).

Microalgae adjust their cellular redox status dynamically in response to fluctuations in their environments, abiotic stress factors in particular (Chisti, 2007). Amongst microalgae, Dunaliella strains are mostly pronounced with their halotolerant and competitive nature (Cifuentes et al, 2001). The genus *Dunaliella* includes around 30 species of which 25 are found in saline water. Several type of microalgae strains from *Dunaliella* genus are distinguished with their ability to produce β-caroten when they are grown under high salt conditions (Spolaore et al., 2006). On the other hand, strain *Dunaliella tertiolecta*

was stated to have potential use of biodiesel feedstock production with a reported oil yield of 36-42% (Tsukahara and Sawayama, 2005).

Several researchers dissected response of *D.tertiolecta* to different environmental factors for suitable oil production for biodiesel feedstock. Influence of photoperiod, light source and intensity, CO_2 concentration, and element manipulation in growth medium have been employed for determination of the best stress factor for triacylglycerol production by *D.tertiolecta* (Tang et al., 2011, Chen et al., 2011). In the literature, Cobalt (Co) was shown to play role in growth and short term Co deprivation was reported to increase neutral lipid content of *D.tertiolecta*. However, to the best of our knowledge, there is no study evaluating carotenoid and neutral lipid production efficiency of *D.tertiolecta* in response to a trace element, Co, deprivation. In this study, we report impact of Co deprivation on neutral lipid and β-caroten production by an indigenous strain *D.tertiolecta* IMCC-37.

2. Methods

Dunaliella tertiolecta IMCC-37 was isolated from a volcanic saline lake, Lake Meke, located in Konya Province with the coordinates of 37° 40' 32''- 37° 41' 33'' north latitude and 33° 38' 36'' - 33° 38' 61'' east longitude. The isolated strain was identified based on genomic information. Genomic DNA was isolated from all algal species via a phenol-chloroform method (Chomczynski and Sacchi, 1987) on a pellet obtained by centrifugation of 10 mL of algal culture at the late-log phase. DNA amplification from genomic DNA containing a partial 18S ribosomal RNA region was performed by PCR using the following primers: Forward: 5'-ATTGGAGGGCAAGTCTGGT-3'and Reverse: 5'-ACTAAGAACGGCCATGCAC-3'. Same primers were used for Sanger sequencing and nucleotide sequences were analysed on NCBI database and BLAST results were used for identification of the strain.

D.tertiolecta IMCC-37 strain was grown in 50ml medium in 100ml flasks under continuous light (150PAR) on an orbital shaker with a 120rpm speed under 28°C temperature. Modified Jhonsons medium (with 20% salt concentration) was used as control and $CoCl_2$ was not included in the growth medium for Co deprivation. Microalgae were cultivated under defined conditions for 1 month and growth was monitored. Besides that, photosynthesis and respiration activity, chlorophyll, carotenoid contents, neutral lipid, triacylglycerol and carbohydrate levels were measured after 15 days of incubation period which is defined as exponential growth phase for the strain *D.tertiolecta* IMCC-37.

Growth was recorded by measuring absorbance of the culture at 680nm. Photosynthesis and respiration efficiency of experimental groups were measured as a function of oxygen consumption with a Clark-type oxygen electrode system (Hansatech Oxytherm, Hansatech Ins. Ltd., Norfolk, U.K.) at liquid phase under light and dark conditions. Chlorophyll and carotenoid content of cells were measured as described by Jeffrey and Humphrey (1975). HPLC analysis of β-carotene was performed as suggested by Fazeli et. Al., (2006). Neutral lipid staining was performed using Nile Red as described by Elsey et al. (2007). Triacylglycerol and carbohydrate levels were measured by Fourrier transform infrared (FT-IR) spectroscopy measurement. For FT-IR measurement, cells were concentrated on a 96 well silicon microplate and oven-dried for 45 min to form homogeneous thin films (Dean et al., 2010). FTIR spectra were recorded using a Nicolet 6700 Research FT-IR Spectrometer (Thermo Scientific). The bands were assigned to specific molecular groups on the basis of biochemical standards and published studies as previously described (Movasaghi et al., 2008). FTIR peak values were of particular interest which were attributed to ester group (C=O) vibration of triglycerides (1744 cm^{-1}), carbohydrate bands (1200–950 cm^{-1}) and amide I absorption (1652 cm^{-1}).

The final data of each experimental group in this article are mean values represented by at least six replicate samples. Standard errors and t-tests (two tails, pair type) with significance criteria of 0.05, 0.01, or 0.001 are used to assess significance.

3. Results and Discussion

Being an integral component of vitamin B12, Co is stated to play a biolimiting role in the oceans (Bruland 1983). Co has been shown to be an important micronutrient for *Dunaliella tertiolecta* growth (Chen et al., 2011). Chen et al., (2011) reported that Co deprivation causes remarkable decreases in

growth starting from third day of deprivation; however, in this study, Co deprivation did not cause a significant change in growth during 30 days of incubation period (Fig. 1).

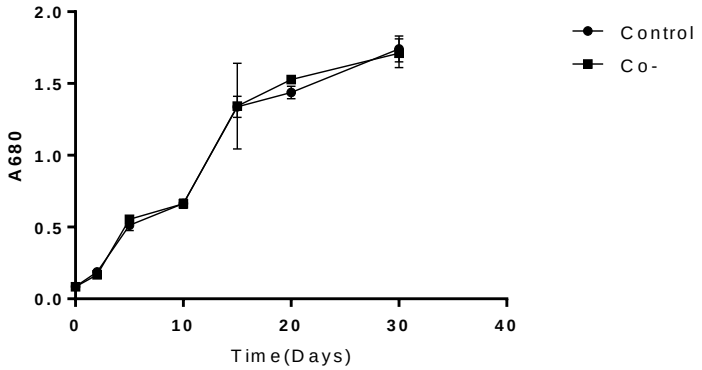

Fig. 1. Growth efficiency of *D.tertiolecta* IMCC-37 under Co deprivation.

Photosynthesis is of vital importance for microalgae in order to supply energy demand to sustain metabolism. Photosynthetic efficiency of microalgae is predominantly reflected by the chlorophyll and carotenoid levels and microalgae need to keep their chlorophyll and carotenoid levels in a balance for an efficient utilization of carbon sources (Zhang et al. 2002). Our results showed that photosynthesis activity of Co-deprived *D.tertiolecta* cells increased as supported by increase in Chlorophyll-a and Chl-a/b ratio. (Fig. 2).

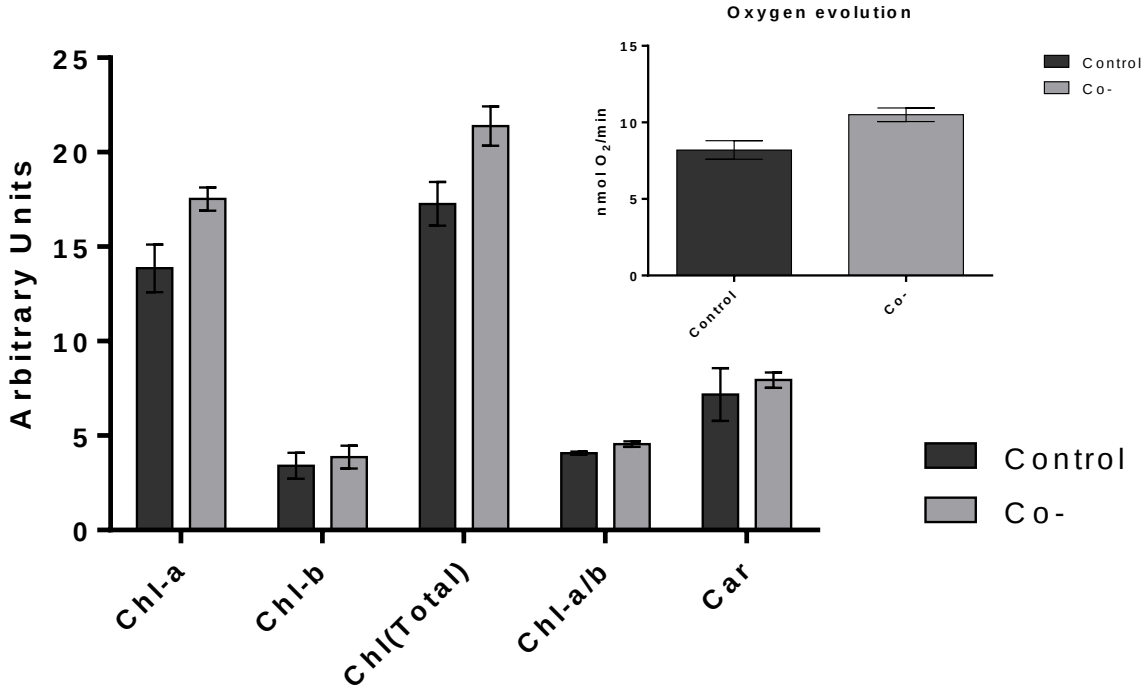

Fig. 2. Level of photosynthetic pigments and photosynthetic activity (inset figure) of *D.tertiolecta* grown under Co-deprivation for 15 days.

Slight increases in oxygen evolution activity and chlorophyll levels in *D.tertiolecta* refers that anabolic activity is increased. Increased anabolic activity would either lead increased cell growth or production of proteins, carbohydrates or lipid based biomolecules. There was no significant difference in growth (Fig.1). Thus, neutral lipid and carbohydrate levels of 15 days old controlled or Co-deprived *D.tertiolecta* cells were analysed. Triacylglycerol levels increased over 27% as supported by 11% increase in neutral lipid content (Figure 3). Increase in neutral lipid content of *D.tertiolecta* was reported before (Chen et al., 2011).

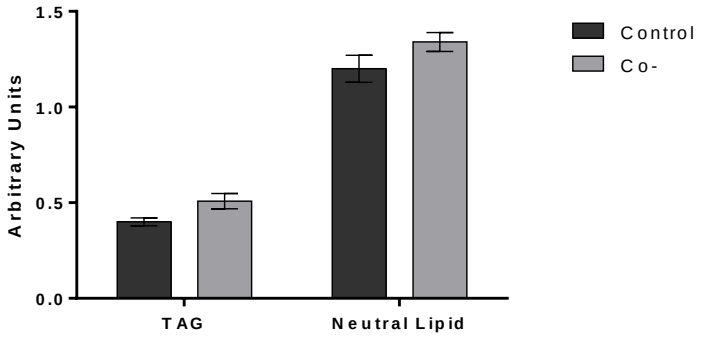

Fig. 3. Level of TAG and neutral lipids in controlled and Co-deprived *D.tertiolecta* cells of 15th day incubation. Values expressed for TAG levels are values from 1744/1652nm wavelength obtained by FT-IR measurement. Neutral lipid levels were defined as fluorescence intensity at 570nm emission wavelength for Nile Red dye.

Halophilic Dunaliella strains are most pronounced for their ability to produce β-caroten under stress conditions (Raja et al., 2007). In this study, carbohydrate level increased over 13% as a response to 15 days Co-deprivation (Fig. 4). Even if there was no significant change in total carotenoid production, β-caroten production increased over 14% at the end of 15days of incubation period (Figure 4).

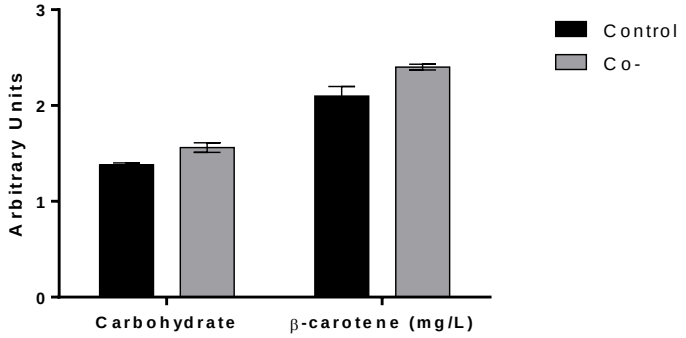

Fig. 4. Level of Carbohydrate and β-caroten in controlled and Co-deprived *D.tertiolecta* cells of 15th day incubation.

In this study, Co deprivation was found to induce neutral lipid and β-carotene production of *D.tertiolecta* as measured on exponential growth phase. Production of β-carotene by *D.tertiolecta*, as well as other halotolerant Dunaliella strains, was reported to increase in response to a stress factor such as high salt conditions (Fazeli et al., 2006). On the other hand, Co deprivation was shown to induce a short term lipid production while causing decrease in growth of *D.tertiolecta* (Chen et al., 2011). Even if our data supports Chen et al (2011) for increased neutral lipid production but we did not observe any significant change in growth. This study shows that, Co deprivation induce β-carotene and neutral lipid production by *D.tertiolecta* and it does not cause any significant change in growth. Increase in photosynthesis

activity and photosynthetic pigment levels refer to increased metabolism which is not reflected by a change in growth but by increase in metabolites such as lipids and carbohydrates.

4. Conclusion

A halotolerant microalga, *D. tertiolecta* is a remarkable candidate for biodiesel feedstock, triacylglycerol, production. This species is not only pronounced for its high lipid content but β-carotene content of this species is also at a considerable level. Thus *D.tertiolecta* possess a potential to be utilized for both triacylglycerol and β-carotene production. In this study, Co deprivation did not cause significant change in growth while significant increase of neutral lipid and β-carotene level of the cells was observed, A major conclusion of this study is that Co deprivation can be used as a potential tool for increasing both triacylglycerol and β-caroten production by *D.tertiolecta*.

Acknowledgements

This study was supported by a grant from the Scientific and Technological Research Council of Turkey (Project# 112Y029).

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