PHOTOLYASE PRODUCTION DURING CAROTENE INDUCTION IN *Dunaliella salina* GROWTH UNDER DIFFERENT LIGHT CONDITIONS FOR COSMETIC APPLICATION

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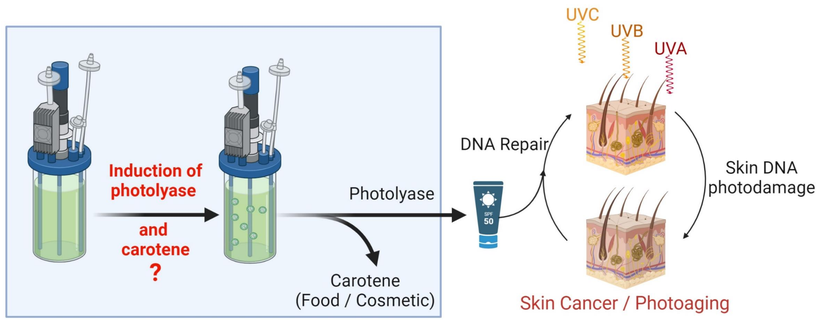
Introduction

Photolyase is an enzyme that repairs DNA damage caused by ultraviolet radiation. Thus, photolyase is employed to develop photoprotective lotions or creams to reduce erythema, skin cancer, and photoaging caused by excessive exposure of skin to the sun. The main source of commercialized photolyase is the cyanobacterium *Synechococcus* spp[1]. Nevertheless, microalgae that are produced industrially could be new sources of photolyase, such as *Dunaliella salina*, which is rich in carotenoid compounds that have several bioactivities for human health. Thus, are used in the cosmetics and foods industries.

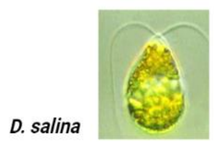
The effect of the different light spectrums during *D. salina* photoautotrophic growth triggers a series of molecular and physiological changes, which can be used biotechnologically to obtain a higher amount of carotenoids, mainly lutein and β-carotene[2]. Also, *D. salina* produces photolyase as part of its DNA repair mechanism. Carotenes and photolyase are induced in *D. salina* by different light types during the culture. Nevertheless, it is unknown the levels of photolyase during the induction of carotenes.

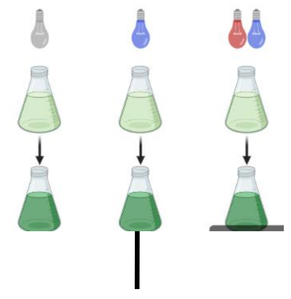


Therefore, we evaluated the activity level of photolyase and carotene profile in *D. salina* culture under White-LED (light emitting diode), Blue-LED, and Blue/Red-LED. For this, photoautotrophic growth of both microalgae was performed under different light types. In addition, the induction of carotene was performed with UVA radiation after photoautotrophic growth under White-LED. For all the experiments, biomass, protein, photolyase activity, photolyase expression, and carotenoid profile were assayed.

Methodology

Microalgae culture





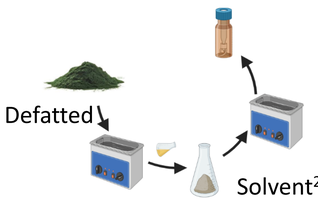
Sampling (centrifugation)
- Exponential phase
- Stationary phase

UVA Induction

Extraction

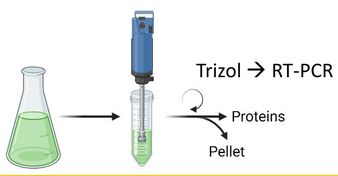
Carotenoids / Chlorophylls

Phenolic compounds



[1]MetOH:TBME (80:20, v:v), [2]Methanol (100%)

Protein extraction and mRNA



Characterization

Total Carotene and Chlorophyll, Antioxidant Capacity

ORAC-FL method, Total Carotene and Chlorophyll (665nm, 652nm, 470nm)

Carotene Characterization

RP-UHPLC-DAD-ESI-MS

Photolyase activity

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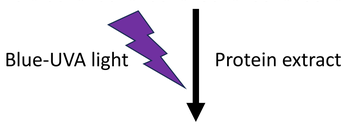

TTTTTTTTTTTTTT

Increase A_{260nm}

Figures and Tables

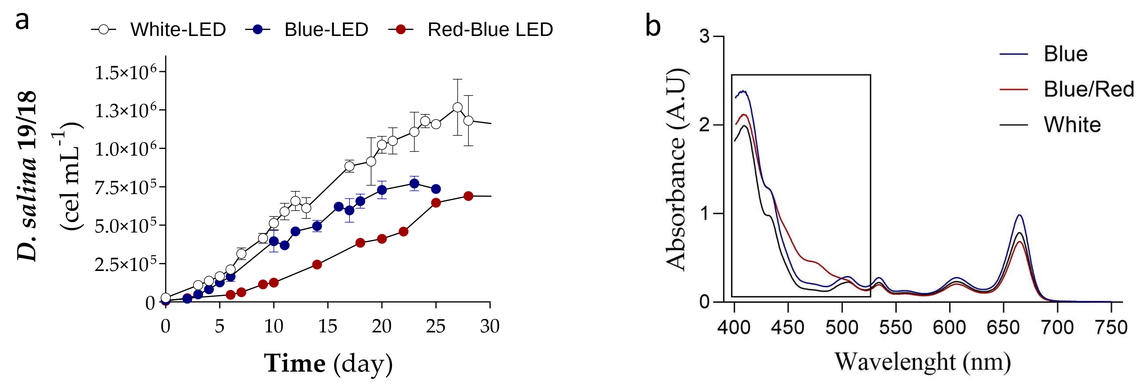

Fig. 1 Growth curve of *D. salina* under different LED lights (100 µmol $m^{-2}s^{-1}$) (a) and spectrogram of acetone extract (b).

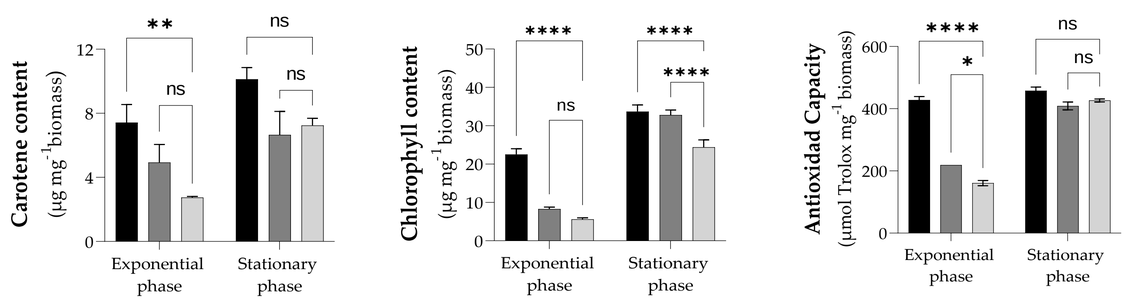

Fig 2. Total Carotene and Chlorophyll Contents, and Antioxidant capacity at two growth phases of *D. salina* under different LED lights (100 µmol m^{-2} s^{-1}). Black bar: Blue LED; Dark grey: White LED; and Grey: Red-Blue LED

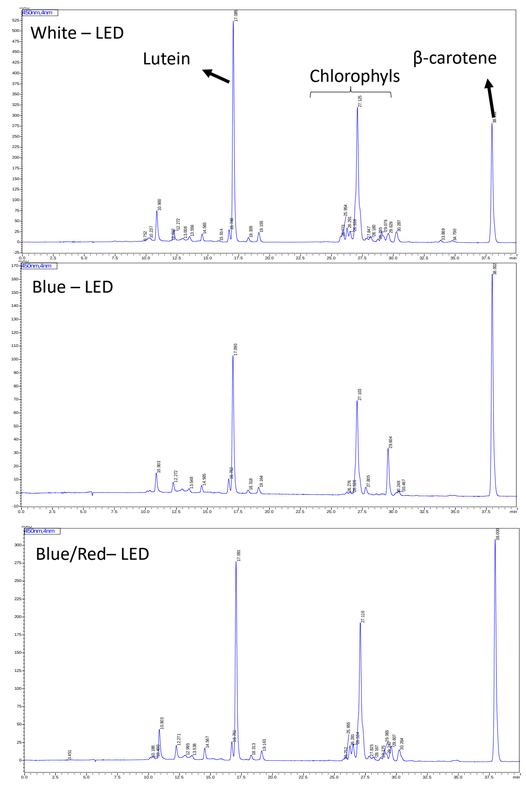

Fig 3. Chromatogram of microalgae MetOH/TBME extracts measured at 450nm. Identification of compounds by Mass spectrometry.

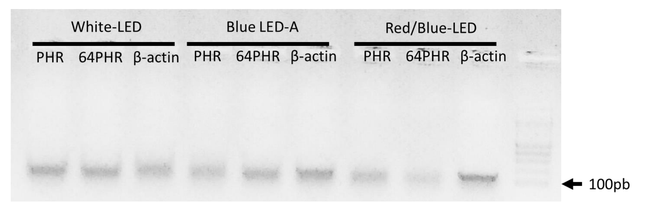

Fig 4. PCR of *D. salina* cDNA with primers designed to PHR, 6,4-PHR and 18S.,

Table 1 Expression level (RT-PCR) of two types of photolyase (PHR and 6,4-PHR) in *D. salina*

Light type	PHR	6,4-PHR
White LED	1.08	1.13
Blue LED	0.77	0.87
Red/Blue LED	0.73	0.31

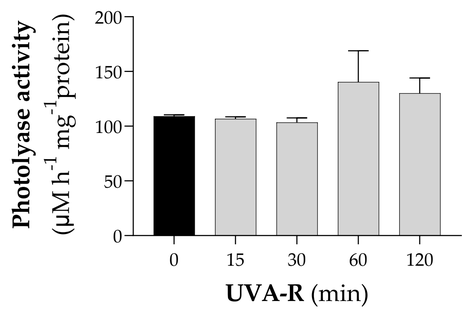

Fig 5. Photolyase activity during the induction of carotenes production on *D. salina*

Results and Discussion

D. salina had a higher growth rate with White-LED, followed by Blue-LED, and Blue/Red-LED (Fig. 1). This result agrees with other studies[3]. Chlorophylls and carotenes were higher at the stationary growth phase. At this phase, the Blue-LED used for the photoautotrophic growth stimulated the biosynthesis of total chlorophylls and carotenes. In addition, degradation metabolites of chlorophyll (pheophytins) were detected by mass spectrometry indicating a high turnover. Therefore, Blue-LEDs stimulate light-harvesting molecules synthesis to overcome the reduced photosynthetic light, which decreases its growth rate. Also, the antioxidant capacity of the acetone extract correlates with the levels of carotene and chlorophylls (Fig. 2).

During the growth of *D. salina*, lutein (and their isomers) and β-carotene were the main compounds with all LEDs. In addition, zeaxanthin, violaxanthin, and neoxanthin were higher with Blue-LED followed by White-LEDs. Overall, the photoautotrophic culture of both microalgae with blue light showed a higher amount of bioactive compounds used during the production process for nutraceutical applications.

Furthermore, photolyase expression was higher when White-LED was used (Fig. 4 and Table 1). Despite photolyases are induced by Blue lights, the growth under continuous Blue-LED didn't increase its expression, neither when complemented with Red-LEDs. Thus, White-LED was preferred to favor the growth of *D. salina*. The induction of photolyase was performed at the beginning of the stationary phase with UVA-R to favor the production of β – carotene[4]. The levels of photolyase activity increased by 35% in comparison to the non-irradiated culture, similar to the cyanobacterium *Synechcoccus* spp.[1]

Conclusion

Variations of the light wavelength during the photoautotrophic culture of *D. salina* increase the production of bioactive compounds. Nevertheless, focus on the correct balance between biomass growth and bioactive compound biosynthesis in the microalgae must be optimized. These results support the use of *D. salina* as a source of photolyase for cosmeceutical applications. Moreover, the development of sequential extractions of photolyase and carotenes could favor a biorefinery bioprocess.

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