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Carotenoids bioproduction by two psychrophilic microalgae *Sphaerocystis sp.* and *Pleurastrum sp.*

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Abstract

Microalgae-derived carotenoids have found a wide range of applications across various industries including food, feed, cosmetics, and pharmaceuticals. Researchers have been seeking microalgae strains that can withstand or adapt to diverse conditions. Among these, microalgae that have adapted to cold climates, known as 'psychrophilic microalgae', hold particular promise. They are potential sources of valuable carotenoids, such as astaxanthin and canthaxanthin. Despite the potential they hold, there is a noticeable scarcity of data on these specific microalgae. This gap in knowledge underscores the need for further research and exploration. As such, the objective of this thesis project was to enhance the accumulation of carotenoids in two specifically selected psychrophilic strains, Sphaerocystis sp., and Pleurastrum sp. These strains have been effectively adapted to our laboratory settings, which promoted cell growth and carotenoid accumulation and extraction.

Keywords: psychrophilic microalgae, cultivation and stress, carotenoids, astaxanthin, canthaxanthin antioxidant

I. INTRODUCTION AND CONTEXT

During the melting season in polar and alpine regions (spring and summer), psychrophilic microalgae are exposed to harsh conditions such as extensive solar irradiation (3000 PAR μ mol/m2/s), elevated temperatures (up to 18° C), and low nutrient availability [1]. In response to difficult circumstances, the microalgae exhibit an impressive capacity to accumulate "stress carotenoids" in shades of red, orange, or yellow linked to significant molecules such as astaxanthin, canthaxanthin, and lutein. These carotenoids are some of the most carotenoids synthesized by the microalgae under stress conditions [2]. It is noteworthy that psychrophilic microalgae carotenoids not only possess strong antioxidant properties but also provide a means of photoprotection, acting as a barrier against the harmful effects of intense sunlight. The value of these carotenoids is rooted in their capacity to counteract the detrimental effects of oxidative stress, which has been linked to various chronic illnesses [3].

The colder and less sunny climate prevalent in Northern Europe could provide an ideal zone for the outdoor cultivation of coldadapted microalgae. It could lead to significant reductions in energy consumption and production costs, making the process more sustainable and economically viable [4]. This innovative approach could pave the way for a new era in the utilization of microalgae, transforming them into efficient, natural factories for carotenoid production. This is particularly relevant as most natural astaxanthin, which is produced by the mesophilic Haematococcus pluvialis, which accumulates up to 4-7% (m/m) of its total dry mass, is currently associated with high energy consumption and production costs. Moreover, recovering carotenoids from microalgae biomass is a challenging task due to the presence of a rigid cell wall, which obstructs the release of intracellular components. Nonetheless, there are various assisted extraction treatments available, such as bead beating, ultrasounds, and pulsed electric fields in adapted solvents, that have been proven efficacious in enhancing carotenoid extraction from microalgae. These treatments aid in disrupting the cell wall and releasing the intracellular components, ultimately increasing the yield of carotenoids [5].

II. MAIN FINDINGS

In this research work, the culture process was carried out in two stages, referred to as green and red, with meticulous monitoring of parameters such as temperature, lighting, and suitable medium culture BBM (Bold Basal Medium). The project has demonstrated encouraging results, even at the relatively small







flasks scale and a 6-liter photobioreactor. Firstly, the transitions between different cell stages have been successful, indicating healthy growth and development of the microalgae at a below temperature of 12 °C, light intensity $< 100 \mu mol/m^2/s$, and 1% of CO₂. The growth rate achieved was between 0.1 and 0.2 day ¹, which is close to the result of reference work by T. Leya of 0.2 day⁻¹. Secondly, the pigmentation of the culture, a key indicator of carotenoid production, has shown positive signs during the adaptation to various abiotic stresses. These stresses included azote and phosphor deficiency, high salinity (60 g/L), and high light intensity (225-325 µmol/m²/s), applied alone or in combination. Lastly, while psychrophilic microalgae are less productive, they have shown a great diversity of pigments by high-performance liquid chromatography such as chlorophylls a and b, astaxanthin, canthaxanthin, and lutein. Extracting these carotenoids was facilitated with the help of mechanical treatment. The study showed that psychrophilic microalgae responded positively when subjected to bead beating in solvents such as ethanol. The total content of carotenoids varied between 2 to 6 mg/g of dry mass in ethanol.

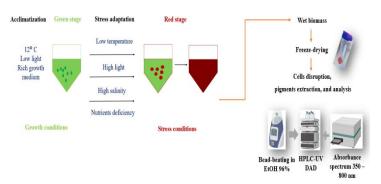


Figure 1: Experimental settings for the cultivation of psychrophilic microalgae, carotenoid production, and extraction method.



Figure 2: *Sphaerocystis sp.* cells undergo a stress period of azote deficiency and high light, resulting in a color shift from green to reddish-orange when observed under a microscope (X400).

III. CONCLUSION AND PERSPECTIVES

The psychrophilic strains, Sphaerocystis sp. and Pleurastrum adapted to sp. were our laboratory settings to grow and accumulate carotenoids. Results are promising as both microalgae have demonstrated the ability to accumulate valuable carotenoids, including astaxanthin and canthaxanthin, during periods of stress. The obtained results were close to the reference publication [2]. However, psychrophilic microalgae be productive tend to less than mesophilic Haematococcus pluvialis. These findings are a solid basis for further optimizing the process to increase the production of biomass and yield of targeted carotenoids from the selected strains.

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