A method for the purification of analytical grade phycocyanin from *Arthrospira platensis* for biotechnological purposes

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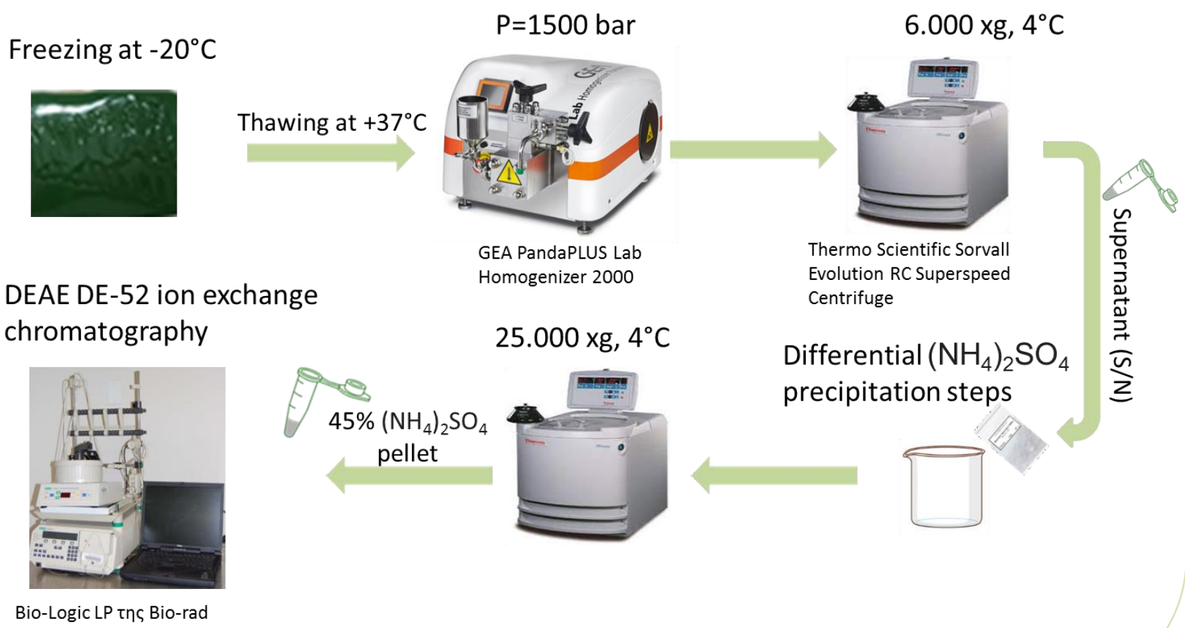
INTRODUCTION

Phycobiliproteins (PBPs) are light-harvesting pigment complexes found in various groups of cyanobacteria, such as *Arthrospira platensis*, also known as *Spirulina*. The main PBPs are phycocyanin, allophycocyanin, and phycoerythrin, which composes larger protein complexes called phycobilisomes (PBSs) functioning that harvest solar energy from visible spectrum in a long range of 450 to 670 nm. Phycocyanin (C-PC) is a hydrophilic fluorescent molecule with a natural bright blue color and displays anti-oxidant and anti-inflammatory properties. C-PC exhibits a large stoke's shift as it absorbs light at 620nm and emits at 642nm. Currently, it is widely used in the food and cosmetics industries as a food-grade, bio-compatible, hypoallergic colorant, in various biotechnological applications as a fluorophore and in health applications as a label in FACS analysis or as a medical device in microsurgery.

The aim of the present study is the purification of analytical grade phycocyanin and the development of methodologies for linking C-PC to certain biomolecules in order to use it as a fluorescent probe. Our study highlights the usefulness of C-PC for biotechnological applications.

MATERIALS AND METHODS



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RESULTS

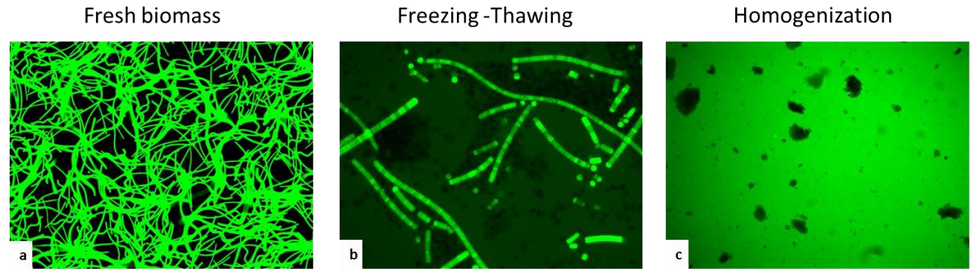

***Fig. 1. Arthrospira plantesis* (*Spirulina*) filaments under fluorescence microscope Zeiss AXIO Scope.A1 using different cell disruption techniques**. a) Sample was taken from fresh biomass without processing. Magnification 100X. b) Sample biomass after freezing (-20°C) – thawing (37°C) treatment. Magnification 400X. c) Sample from *Arthrospira plantensis* after homogenization at 1500 bar. Magnification 100X.

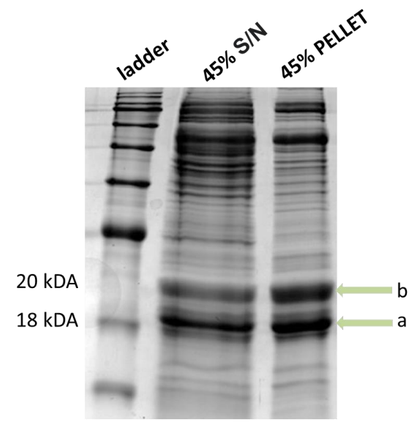

Fig. 2. Electrophoretic analysis of protein profile after differential precipitation of $(NH_4)_2SO_4$. The prepicipation was performed at 45% $(NH_4)_2SO_4$ after 35% $(NH_4)_2SO_4$ precipitation cut followed by 12% SDS-PAGE. a, C-PC *alpha* subunit, b, C-PC *beta* subunit.
Ladder: Prestained pink protein ladder from Nippon Genetics.

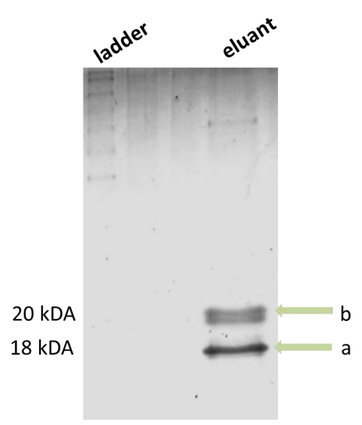

Fig. 3. Electrophoretic analysis of eluted proteins after DEAE DE-52 ion exchange chromatography (12% w/v SDS-PAGE). a, C-PC *alpha* subunit, b, C-PC *beta* subunit. Ladder: Prestained pink protein ladder from Nippon Genetics.

DISCUSSION

Our study showed that:

- ✓ A single cycle of homogenization at 1500 bar was the optimal procedure for efficient cell rupture.
- ✓ A two-step $(NH_4)_2SO_4$ cut (40% and 45% saturation) was the ideal sample preparation for subsequent ion exchange chromatography.

In conclusion, we have achieved the purification of analytical grade phycocyanin ($A_{620}/A_{280} > 4.0$).

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