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Method Article

A simple and fast method for extraction and quantification of cryptophyte phycoerythrin

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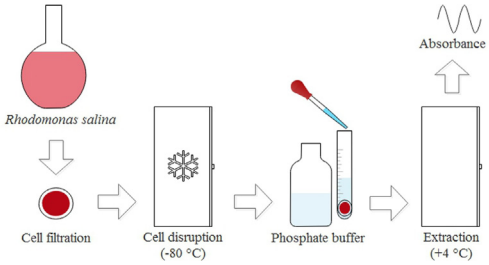
GRAPHICAL ABSTRACT



ABSTRACT

The microalgal pigment phycoerythrin (PE) is of commercial interest as natural colorant in food and cosmetics, as well as fluoroprobes for laboratory analysis. Several methods for extraction and quantification of PE are available but they comprise typically various extraction buffers, repetitive freeze-thaw cycles and liquid nitrogen, making extraction procedures more complicated. A simple method for extraction of PE from cryptophytes is described using standard laboratory materials and equipment. The cryptophyte cells on the filters were disrupted at −80 °C and added phosphate buffer for extraction at 4 °C followed by absorbance measurement. The cryptophyte *Rhodomonas salina* was used as a model organism.

- Simple method for extraction and quantification of phycoerythrin from cryptophytes.
- Minimal usage of equipment and chemicals, and low labor costs.
- Applicable for industrial and biological purposes.

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Background

Phycoerythrin (PE) is a light harvesting pigment belonging to the phycobiliproteins, which also include phycocyanin, allophycocyanin and phycoerythrocyanin. Phycobiliproteins are found in red algae, cryptophytes and cyanobacteria [1], and are used as natural colorant in food and cosmetics. In particular, phycoerythrin is used as a fluoroprobe for clinical and biological analysis due to its high fluorescence [1].

The cell content of PE in microalgae depends on species and cultivation conditions. Generally, microalgae sustain cellular growth/metabolism during nitrogen limitation by degradation of phycobiliproteins [2,3] which are nitrogen-rich. As an example, a study by Eriksen and Iversen [4] showed that nitrogen-sufficient cells of the cryptophyte *Rhodomonas* sp. were red and contained PE, while nitrogen-limited cells were green and without detectable amounts of PE. Also, the cell content of PE in *R. salina* was lower during nutrient limited cultivation compared to nutrient excess in a study by Vu et al. [5]. According to Kathiresan et al. [6], the content of PE in the red microalgae *Porphyridium purpureum* depends not only on nitrogen but on various specific macro nutrients. Light intensity and temperature can also affect the cell content of PE as demonstrated by Chaloub et al. [7] where PE in *Rhodomonas* sp. increased at low light intensity (15 μmol m^{-2} s^{-1}, 12:12 light: dark cycle) combined with increased temperature (26 °C). Thus, quantification of the microalgal cell content of PE has relevant purposes such as being a proxy for the nutrient status during cultivation, and optimizing cultivation conditions to yield a higher cell content of PE.

The extraction efficiency of PE from microalgae depends on the rigidity of the cell wall, if present. The most suitable cell disruption method is therefore species dependent [8]. Cryptophytes do not possess a cell wall but a periplast of thin and fragile rectangular plates underneath the plasma membrane, which is very fragile (see references in Goldman and Dennett [9]) and easily disrupted. Numerous methods for extraction of PE are available but they are based on various species and comprise unnecessary chemicals, working steps and equipment for the extraction of PE from species without a cell wall [6,10,7]. The methods are often too comprehensive and time consuming for simple purposes such as comparing the cell content of PE whether it is between species/strains or between different cultivation conditions. For such comparisons it is beneficial with a simple and low labor cost method to obtain fast results.

This paper describes a simple and fast method for extraction and quantification of PE from the cryptophyte *R. salina* using only few materials and equipment easily available in standard laboratories at a low labor cost.

Method details

Materials

- Culture of *Rhodomonas salina*
- Whatman™ GF/C filter (0.2 μm)
- Pyrex glass vials
- Phosphate buffer (0.1 M, pH 6.7)
- Pasteur glass pipettes
- Syringe with 25 mm syringe filter (0.2 μm cellulose acetate membrane)
- Plastic cuvettes