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Proceeding Paper

Phycoerythrin from *Porphyridium purpureum*: Highly Efficient Extraction, Purification, and Microencapsulation for Food Applications †

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Abstract: This study presents a characterization of phycoerythrin (PE) derived from *Porphyridium purpureum*, a marine microalga. *P. Purpureum* was grown and phycoerythrin was extracted and concentrated to 0.3 mg/mL and a purity index of 6.05. Subsequently, PE was evaluated for its antiproliferative activity against the HEPG2 cell line, a representative model for hepatic cancer. In addition, the study introduced an electrospray-assisted technique to encapsulate the pigment. The results revealed that the pigment exhibited remarkable antiproliferative activity, and an encapsulation efficiency of 99% was achieved. The study serves as a foundation for further exploration and development of *P. purpureum*-derived phycoerythrin as a versatile and valuable bioactive compound.

Keywords: phycoerythrin; phycobiliproteins; *Porphyridium purpureum*; bioactive compounds; anticancer activity

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1. Introduction

Phycoerythrin is a red fluorescent pigment, belonging to the phycobiliprotein family. These proteins are the main component of the light-harvesting complexes in red algae but can also be found in cyanobacteria [1]. Phycoerythrin has been used as a fluorescent marker in flow cytometry [2]. However, it has also been shown to present antioxidant and anticarcinogenic activity [3–5]. These properties have made phycoerythrin an interesting compound to be studied due to the potential applications it may have in different industries, such as food, biomedicine, or cosmetics [6].

Unfortunately, practical applications for phycoerythrin have been limited because it is a pigment sensitive to environmental factors, such as changes in pH, light or temperature [7]. Because of this, encapsulation has emerged as an attractive alternative to address these challenges [8]. Encapsulation consists of trapping a compound of interest in a protective matrix, to protect it from external factors and increase its stability [9].

This research aims to shed light on the potential of phycoerythrin as a highly valuable bioactive compound and the promising role that microencapsulation can play in unlocking its full potential.

2. Materials and Methods

2.1. Microalgae Growth

The marine strain *Porphyridium purpureum* (UTEX LB 2757) was procured from UTEX (UTEX, Austin, TX, USA) and cultivated in F/2 medium. This cultivation process was carried out with control measures, including a 12-h light/12-h dark cycle using LED lamps

that consistently emitted an average intensity of 90 μmol/m²/s. To maintain optimal growth conditions, aeration was provided via an air pump (HAGEN, 801), ensuring a constant air flow rate of 1 L/m²/min. The culture thrived under these conditions for a 14-day incubation period, preparing the groundwork for subsequent phycoerythrin extraction.

2.2. Phycoerythrin Extraction, Quantification, and Purification

The harvested *P. purpureum* underwent cell disruption through a series of freeze-thaw cycles. Specifically, 1 L of *P. purpureum* was frozen at −80 °C for 4 h, followed by thawing with hot water (30 °C) for 30 min. This process was repeated three times.

Subsequently, the extract underwent two rounds of filtration. The first filtration employed a membrane with a 10 kDa MWCO (Koch Membrane Systems Inc.), and the second filtration utilized a membrane with a 2 kDa MWCO (GE Healthcare, UFP-10-E-6A). After passing through the 10 kDa membrane, the permeate was discarded, and the retentate underwent another filtration step using the 2 kDa membrane.

Phycoerythrin content was determined by measuring absorbance at 564, 592, and 455 nm using a spectrophotometer (Thermo Scientific, Varioskan Flash, Waltham, MA, USA). These absorbance values were then applied to the Beer & Eshel equation (Equation (1)) [10].

$$\text{PE (mg/mL)} = [(OD564 - OD592) - (OD455 - OD592) \times 0.2] \times 0.12 \quad (1)$$

To determine PE purity, absorbance at 565 nm and 280 nm were measured. The absorbance values at 565 nm and 280 nm are indicative of PE and protein concentrations, respectively [11,12]. Purity index was calculated using Equation (2):

$$\text{Purity index} = A_{565}/A_{280} \quad (2)$$

2.3. In Vitro Evaluation of Anticancer Activity

The HepG2 human hepatoma cell line was cultured in DMEM-F12 medium, supplemented with 20% fetal bovine serum (Gibco, Grand Island, NY, USA), and maintained at 37 °C in a humidified atmosphere with 5% CO_2. For the experimental setup, 96-well plates were prepared with 20,000 cells per well.

Various concentrations of phycoerythrin (ranging from 0.05 to 0.30 mg/mL) were utilized in the study. The assessment of antiproliferative activity was conducted after 24 h using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA), following previously established protocols [13].

2.4. Microencapsulation of Phycoerythrin

A 2% *w/v* alginate solution was blended with a PE solution (0.3 mg/mL). A 2% *w/v* calcium chloride solution was separately prepared by dissolving it in distilled water at pH 4.0 (adjusted with 1 N acetic acid) for 20 min.

Microencapsulation was carried out using a Yflow Startup Electrospinning Machine. Solutions containing phycoerythrin and sodium alginate were introduced through a 10 mL syringe at a flow rate of 15 μL/min. The nozzle was positioned 5 cm away from the collector, which contained a 2% $CaCl_2$ solution. The applied voltage differential was 5.4 kV.

2.5. Encapsulation Efficiency

The encapsulation efficiency was determined following the following equation [14]. This calculation involved measuring the initial mass of phycoerythrin added and the mass that remained in the calcium chloride solution (Equation (3)).

$$\text{EE (\%)} = \frac{\text{Mass of PE added at the beginning} - \text{Mass of not coated PE}}{\text{Mass of PE added at the beginning}} \times 100 \quad (3)$$

2.6. Morphological Characterization of Microcapsules

Microparticle morphology was assessed via Scanning Electron Microscope (SEM) utilizing a Carl Zeiss EVO MA 10 instrument. Initially, the microcapsules were affixed to stubs using double-sided carbon adhesive tape. Subsequently, a delicate gold coating was applied to the stubs. A magnification of 100X was used. The acceleration voltage employed was 5.00 kV.

3. Results and Discussion

3.1. Phycoerythrin Extraction

In this study, using the phycoerythrin extraction method by membrane filtration, a concentration of 0.3 mg/mL and a purity index of 6.05 were achieved.

The extraction of phycoerythrin is a process that requires specialized techniques. Throughout research, several strategies have been developed to efficiently isolate this compound. Solvent-based methods involve the use of organic solvents such as acetone or ethanol to dissolve the pigments and proteins present in the algal biomass. On the other hand, aqueous extraction uses water as a solvent to gently extract phycoerythrin. Precise control of temperature and pH during extraction is critical to maintain the stability of the phycoerythrin. Although these techniques are effective, it is important to consider factors such as cost and safety, as some methods involve hazardous solvents. Therefore, the approach employed in this study represents a more environmentally friendly and economical alternative to conventional methods.

3.2. Phycoerythrin Encapsulation

Under the previously mentioned conditions, encapsulation of phycocyanin using sodium alginate demonstrated an 99% (±0.16%) encapsulation efficiency. This suggests that these capsules have great potential for future research aimed at determining whether the stability provided by encapsulation improves or enhances the bioactivity of this phycobiliprotein.

In this study, the alginate microcapsules displayed an approximate diameter of 671 μm, in line with earlier research results [14]. Nonetheless, when alginate alone is employed in the creation of microcapsules, it frequently results in structures featuring highly porous walls, which could potentially result in the loss of the core substance.

The surface, shape, and size of the capsules produced can be seen in Figure 1.

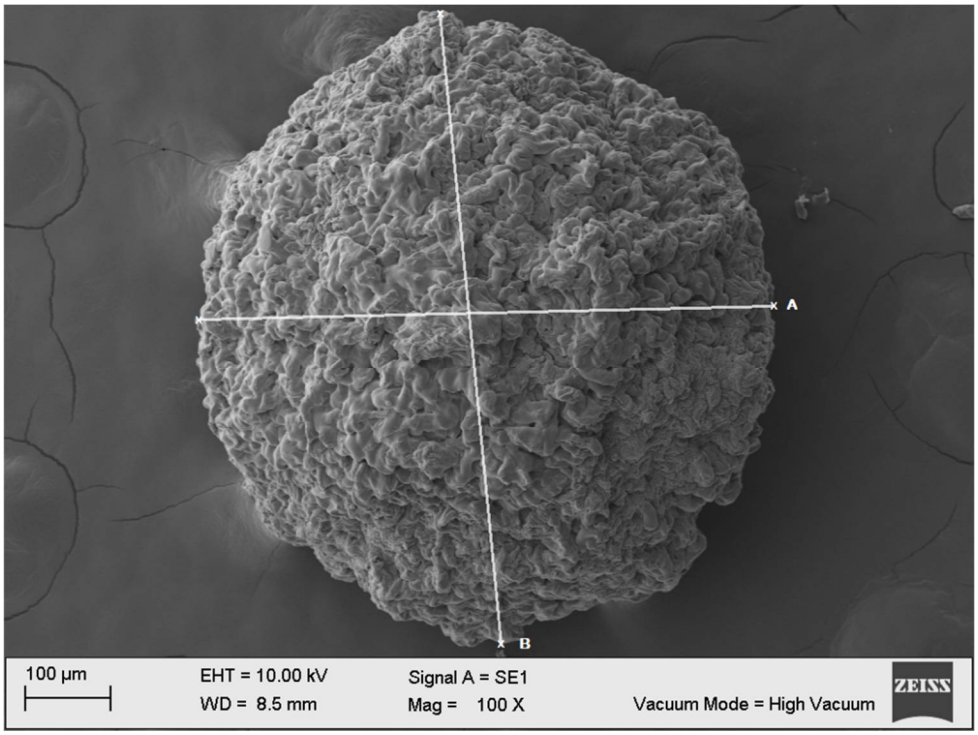

Figure 1. SEM micrograph of microcapsules produced. Segment A is 671.2 µm long, segment B is 744.8 µm long.

3.3. In Vitro Antiproliferative Activity of Phycoerythrin

An in vitro assessment of the antiproliferative activity of phycoerythrin at different concentrations (ranging from 0.05 to 0.30 mg/mL) was conducted against the hepatocellular carcinoma cell line (HepG2). It was observed that the phycoerythrin concentration exerts a dose-dependent effect on cell viability, a finding consistent with previous reports by other authors [15].

The highest anticancer activity was observed at the highest concentration, resulting in a cell viability of 30.71% (±6.18%), while the lowest activity was found at the concentration of 0.05 mg/mL, with a cell viability of 85.73% (±8.14%). These findings align with those reported by other researchers when examining the effect of phycoerythrin on the HepG2 cell line. However, it's worth noting that the phycoerythrin previously studied has been extracted from different species, such as *Microchaete*, *Porphyra yezoensis*, and *Portieria hornemannii* [3–5]. In *Pyropia yezoensis* and *Portieria hornemannii*, the evaluation of the antiproliferative effect was also measured at 24 h. However, in the study carried out with *Microchaete*, the measurement was performed at 72 h. Further studies are needed to determine the antiproliferative activity of phycoerythrin over time.

The antiproliferative effect of phycoerythrin at different concentrations is illustrated in Figure 2.

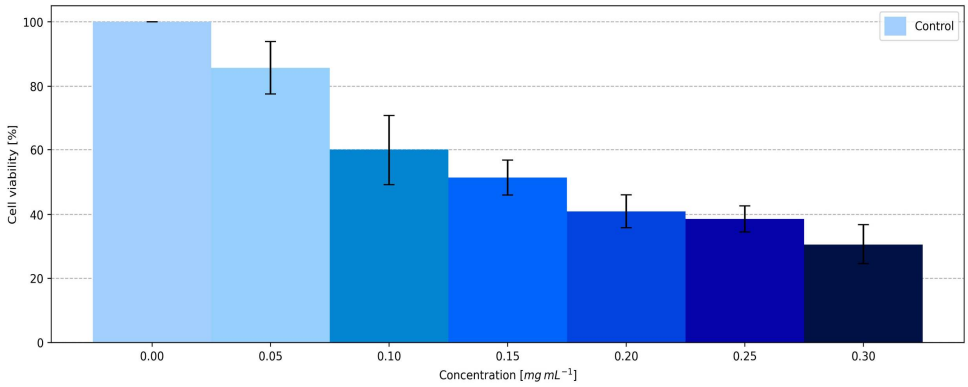

Figure 2. Antiproliferative effect of different concentrations of phycoerythrin.

4. Conclusions

Phycoerythrin was successfully extracted from the microalga *Porphyridium purpureum*, reaching a concentration of 0.3 mg/mL. Subsequently, this phycobiliprotein was effectively microencapsulated using an electrospray-assisted technique with sodium alginate as the encapsulating material. The encapsulation efficiency achieved was 99%, and morphological characterization of the capsules revealed diameters ranging from 600 to 700 nm. When tested against the hepatocellular carcinoma cell line (HepG2), phycoerythrin exhibited significant antiproliferative activity, achieving a cell viability of 30.71% (±6.18%) at the highest evaluated concentration, which was 0.30 mg/mL. This opens further research lines aiming at extending the studies of the biological activity of phycoerythrin and considering the beneficial effects it can have on health, as well as assessing several ways to increase its stability to environmental factors in order to expand its uses in a variety of industries.

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