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Effect of Pb^{2+} on Phycobiliprotein Content of *Spirulina platensis*, an Edible Cyanobacterium

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ABSTRACT. *Among the commercialized microalgae, increasing attention is being focused on Spirulina platensis, because of its multi-nutritional properties and other benefits. Taking into consideration that the chemical composition of an organism could vary with environmental conditions, the present study was focused on the effect of Pb^{2+} on phycobiliprotein content of S. platensis. The test was conducted in Zarrouk liquid medium containing various Pb^{2+} concentrations (0, 1, 10, 30, 50 and 100 μg/mL). At the end of 10 d culture period, the cultures were subjected to analysis of principal phycobiliproteins; phycocyanin (CPC), allo-phycocyanin (APC) and phycoerythrin (PE) contents. The growth of S. platensis was also monitored routinely by counting cells. Though, Pb^{2+} at high concentrations could adversely affect growth (2.29, 37.95, 49.94 and 82.0% inhibitions respectively at 10, 30, 50 and 100 μg/mL), a slight growth stimulation was seen at low concentration (1 μg/mL). Phycobiliprotein contents were also found to have decrease with the increased Pb^{2+} concentration in the medium. The highest reductions (68.4, 65.5 and 83.3%, respectively for CPC, APC and PE) were observed at 100 μg/mL. Results further revealed that reductions in phycoerythrin contents at 50 and 100 μg/mL of Pb^{2+} were remarkably higher than those of the other two pigments. Metal induced changes in the arrangement and structure of the photosystem II, the multiprotein complex localized in the thylakoid membrane could be the possible reason for decreased phycobiliprotein contents at a higher concentration of Pb^{2+}.*

INTRODUCTION

Photosynthetic microorganisms such as microalgae and cyanobacteria have an efficient biological system for harvesting solar energy to produce organic compounds (Vonshak, 1997). Commercialized production of these organisms began with the culture of *Chlorella* in Japan in the 1960s followed by the cultivation of *Spirulina* in Mexico, the USA and China in the 1970s (Radmann *et al*., 2007). In cyanobacteria, the light-harvesting pigments include chlorophyll *a*, carotenoids and phycobiliproteins (Reis *et al*., 1998). The latter are proteins called bilins, found only in cyanobacteria, red algae and cryptomonads (Bermejo *et al*., 2002). Chlorophyll and phycobilins belong to the same family of tetrapyrroles formed by the condensation of eight molecules of aminolevulinic acid

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(Cornah *et al*., 2003). Phycobiliproteins are the major photosynthetic pigments in cyanobacteria (Patil and Raghavarao, 2007). These water-soluble proteins can be divided into three main classes based on their structure: phycoerythrins, phycocyanin and allophycocyanins (Bermejo *et al*., 2003). C-phycocyanin (C-PC), the major component of the phycobiliprotein family exhibits a strong red fluorescence when it is present in native and concentrated form (Patil and Raghavarao, 2007). It has been used as a nutrient ingredient, natural coloring agent, cosmetics (Yoshida *et al*., 1996) and therapeutic agent in oxidative stress-induced diseases (Bhat and Madyastha, 2001). Recently, it has been observed that phycocyanin also has anti-inflammatory and anti-cancer properties (Reddy *et al*., 2003).

The cyanobacteria *Spirulina platensis* is an excellent source of phycocyanin (Silveira *et al*., 2007). The protein fraction of *S. platensis* may contain up to 20% of phycocyanin (Vonshak, 1997). In addition to the high phycocyanin content, this species has used as a multi-nutritional source of healthy food, feed and pharmaceutical products (Anupama, 2000). The species contains some fine compounds such as essential fatty acids, amino acids, antioxidant vitamins, minerals and proteins at relatively high concentrations and thus has been gained increasing attention (Estrada *et al*., 2001). Studies were accomplished evaluating the physical, chemical and nutritional parameters of the cultivation of this species (Costa *et al*., 2003) as well as determining the engineering parameters of the cultivation process (Costa *et al*., 2004).

The chemical composition of *S. platensis*, however, is considerably influenced by growing conditions such as pH, temperature, light intensity and the presence of contaminants (Rafiqul *et al*., 2005), the presence of bicarbonate ions (Costa *et al*., 2002), nitrogen source (Danesi *et al*., 2002), initial biomass concentration (Pelizer *et al*., 2003) and population density (Gitelson *et al*., 1996). Therefore, the study of the response of this species to metal exposure is of particular relevance. The present study deals with the effect of Pb^{2+} on phycobiliprotein content of *S. platensis*.

MATERIALS AND METHODS

Strain, media and culture procedures

The axenic stock cultures of the cyanobacterium, *S. platensis* strain 6 - 1 preserved in our laboratory at Life Science College, Ocean University of China, were used for the experiment. The species was grown in Zarrouk liquid medium (Parada *et al*., 1998) adjusted to pH 7.0 at 25^{o}C. The cultures were gently stirred and illuminated with white light produced by neon tubes at 90 μmol photon $m^{-2}s^{-1}$ with a light/dark cycle of 14:10 hrs. At the exponential growth phase, the algal mass was harvested by filtering through a muslin cloth and washed the pellet was three times with deionized water. The pellets were then resuspended in fresh Zarrouk medium in order to use for the metal treatments.

Chemical regents and analytical methods

Analytical grade chemicals were used throughout the experiments without further purification. A stock solution of Pb^{2+} (1000 μg/mL) was prepared using $Pb(NO_3)_2$ and kept in a refrigerator at 4^{o}C until use. All the working solutions were prepared by properly diluting the stock solution. De-ionized water obtained from a Millipore Milli-Q system was

used throughout the experiment. A spectrophotometer (UV - 2102) was used to measure optical density while pH measurements were made with a pH/ISE meter (model 868). Lead concentrations in the medium were measured using an Atomic Absorption Spectrophotometer (PGENERAL, TAS-986). An ultrasonic liquid processor (SONICS) was used to break algal cells in order to determine pigment contents. All measurements of weight were performed using a digital balance (Sartorins, BS 210 S).

Lead treatments

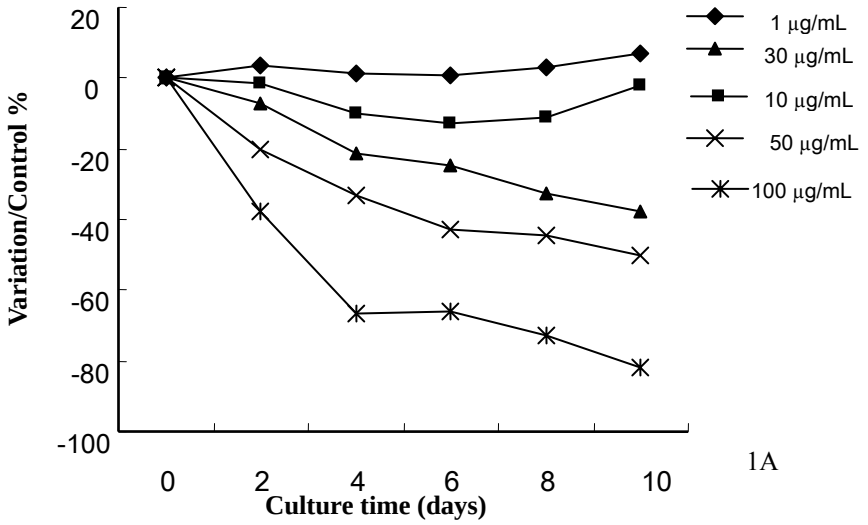
Toxicity assay was carried out using 150 mL Elementary flasks filed with 100 mL of algal suspension. After adjusting the initial Pb^{2+}concentrations (1, 10, 30, 50 and 100 μg/mL), the cultures were continuously homogenized in a rotary shaker at 100 rpm and incubated for 10 days aseptically under the culture condition as described above. The algal cells without Pb^{2+} in the medium served as the control. The starting optical density was set to 0.10 at 560 nm and the pH was adjusted to 7±0.1 with 0.1 M HNO_3 or NaOH to minimize Pb^{2+} precipitation. The experiments were conducted in triplicate and repeated twice to confirm the reproducibility of the results.

Growth and pigment contents

The growth was monitored by direct cell counting using a cell counter (XB-K-25, Shanghai, 0.1 mm deep) under a light microscope (Olympus, Transmission, LM). At the end of 10 day, the cultures were subjected to analyze the pigments. Chlorophyll *a* and β carotene were extracted in 90% acetone and estimated according to Ben-Amotz and Avron, (1983). Phycobiliprotein content was determined spectrophotometrically. Briefly, the cells were filtered through a glass-fiber filter paper (Whatman GF/C, 0.45μm) and meshed with a glass rod in a plastic centrifuge tube after adding 10 mL of 0.01 M Na_2HPO_4 containing 0.15 M NaCl at pH 10. The meshed samples were sonicated for 4 min and centrifuged at 3000 rpm for 10 min. The supernatant was transferred into a separate tube, followed by resuspension of the residue in Na_2HPO_4 solution to repeat the extraction. The samples were made up to a known volume and the optical densities at 652, 615 and 562 nm were measured. The phycobiliprotein concentrations were calculated according to Bennert and Bogorad (1973).

RESULTS AND DISCUSSION

As can be seen in Figure 1A, the culture media with low concentration (1 μg/mL) of Pb^{2+} exhibited better cellular growth than that of the control. However, increase in Pb^{2+} concentration in the media adversely affected on growth. As Figure 1B illustrates, Pb^{2+} at higher concentrations inhibited the growth of *S. platensis* in a dose-dependent manner.



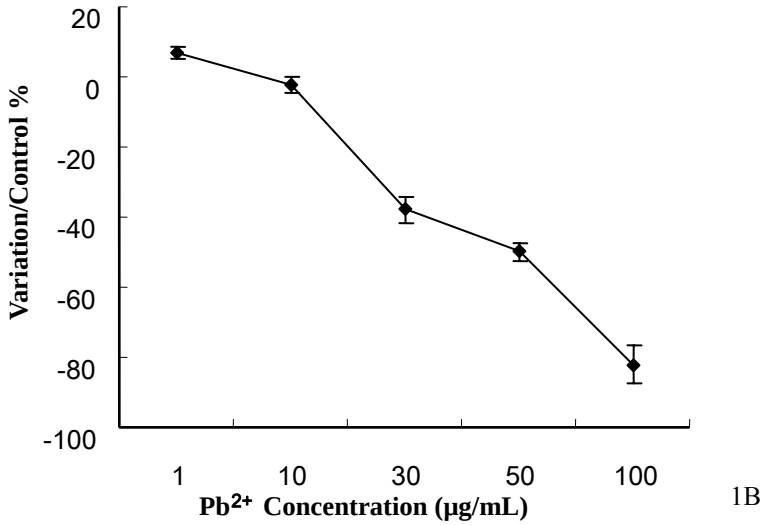


Figure 1. Effect of different concentrations of Pb^{2+} (μg/mL) on growth of *S. platensis* (S_{6-1}).

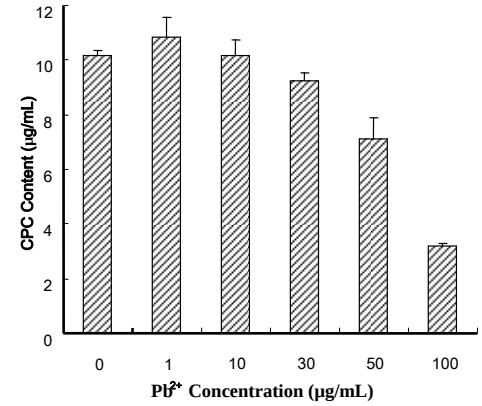
Note: (A) cultured in Zarrouk medium (pH 7.0) under illumination of 90 μmol photon m^{-2} s^{-1} at 25 ± 1°C. (B) Dose-response plot for cell count data at day 10. Mean ± SD, n = 3.

Hong and Shan-shan (2005) reported that high concentration of Pb^{2+} (> 20 μg/mL) caused a large number of *Spirulina* cells to die resulting in growth inhibitions, which is in good agreement with present results. The high toxicity of Pb^{2+} to cyanobacteria was also reported by Heng *et al*. (2004), as they observed a 50% growth inhibition in *Anabeana flos-aquae* at 1 mg/L of Pb^{2+}. The concentration-dependent reduction in the cell count of the present study is also in good agreement with the findings of Fathi *et al*. (2000).

The contents of all three pigments at low concentration (1 μg/mL) were slightly higher (6.9, 7.8 and 3.0%, respectively for phycocyamin (CPC), allo-phycocyamin (APC) and phycoerythrin (PE) than those of the control (Figure 2). Gradual increase in Pb^{2+} concentration in the media has resulted in decreased pigment contents. The highest reductions (68.4, 65.5 and 83.3% respectively, for phycocyamin, allo-phycocyamin and phycoerythrin were observed at 100 μg/mL. Results further revealed that reductions in phycoerythrin contents at 50 and 100 μg/mL of Pb^{2+} were remarkably higher than the other two pigments.

Phycobiliproteins are assembled into particles named phycobilisomes which are attached in regular arrays to the external surface of the thylakoid membrane and act as major light harvesting pigments in cyanobacteria and red algae (Sarada *et al*., 1999). Phycobilisomes consist of allophycocyanin cores surrounded by phycocyanin on the periphery. Phycocyanin is the major constituent while allophycocyanin functions as the bridging pigment between phycobilisomes and the photosynthetic lamella (Gantt, 1981). Metal induced changes in the arrangement and structure of the light-harvesting chlorophyll-protein complex II is common in cyanobacteria (Sersen and Kralova, 2001). Photosystem II (PSII) is a multi-protein complex localized in the thylakoid membrane. It is particularly in the PSII region, on the donor as well as the acceptor that many toxic metals block electron transport (Krupa and Basznski, 1995). Metals cause an inactivation of the PSII reaction center, affecting the rate of carbon dioxide fixation (Surosz and Palinska, 2004). Murthy and Mohanty (1991) have observed that the energy transfer from phycocyanin (PC) to chlorophyll *a* (Chl *a*) in the intact cells of the *S. platensis* was inhibited by heavy metals.

Surosz and Palinska (2004) observed that the cellular components of the thylakoid membranes of *Anabaena flos-aquae* were deteriorated by increased copper and cadmium concentrations. Similar observations in the same species were also made by Heng *et al*. (2004) for lead and cadmium and Rangsayatorn *et al*. (2002) for lead and Zink. In fact, alterations in the ultrastructure of thylakoid were proved to be quite general regarding metal toxicity to cyanobacteria. The deterioration of the thylakoid membranes implicates a loss of photosynthetic activity and pigments. A decrease in thylakoid surface of metal-treated cells indicated a loss of photosynthetic potential, both by a decrease of the proteiaceous lamellae and by a decrease in cells' chlorophyll content. This eventually could affect synthesis of phycocyanin and carotenoid as reported by Atri and Rai (2003), who studied the effect of cadmium on three different cyanobacterial genera.



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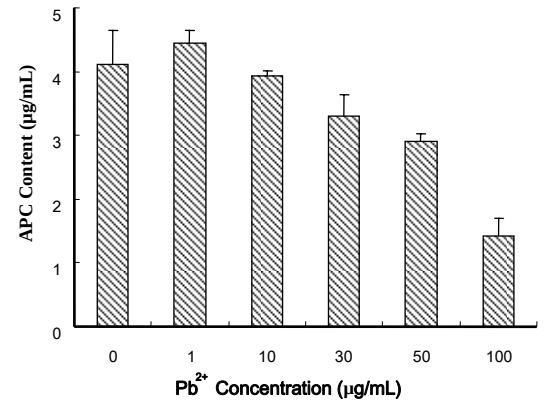

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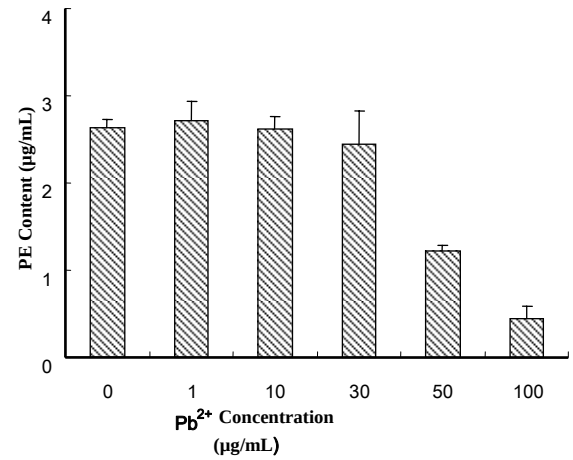

2C

Figure 2. Effect of different concentrations of Pb^{2+} (μg/mL) on phycocyanin (A), allophycocyanins (B) and phycoerythrins (C) content of *S. platensis* (S_{6-1})

Note: *S. platensis* (S_{6-1}) cultured in Zarrouk medium (pH 7.0) under illumination of 90 μmol photon m^{-2} s^{-1} at 25 ± 1°C Mean ± SD, n = 3.

Trapping of metals by polyphosphate bodies may be a significant means by which the metals are taken up by aquatic organisms (Surosz and Palinska, 2004). Pettersson *et al*. (1988) and Jensen *et al*. (1982) found that polyphosphate granules represent a significant binding site for many different heavy metals and suggested the hypothesis that the binding of metals is related to a decrease in the mobilization of the polyphosphate granules, which leads to phosphorus starvation. Surosz and Palinska (2004) observed large cyanophycin granules in copper treated *A. flos-aquae* and suggested that it may be correlated to the phosphorus limitation. Limitation of phosphorus also has an effect on the phycobiliprotein content in cyanobacteria, as well as on chlorophyll *a*. Due to phosphorus depletion, cell constituents such as nucleic acids cannot be regularly synthesized, thus the biosynthesis of proteins and enzymes is blocked and a serious impediment in cell growth can be resulted in. Metal concentrations in polyphosphate granules may have a deleterious effect on the photosynthetic apparatus, which leads to a decrease in supply of adenosine triphosphate and $NADPH_2$ (Surosz and Palinska, 2004).

CONCLUSIONS

The results obtained from the present study provide information about the inhibitory effect of Pb^{2+} on growth and phycobiliprotein content of *S. platensis*. This indication may have been caused by the deterioration of the thylakoid membranes as suggested by previous studies. However, a better understanding of the mechanism requires further study of the effect of Pb^{2+} on pigment synthesis pathway of the cyanobacteria.

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