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Regulation of Phycocyanin Development by Phenolic Compounds in the Cyanobacterium *Anabaena* sp. PCC 7120

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Phenolic compounds are manufacturing by-products commonly found in industrial wastewater. The toxicity of high level phenolic compounds in wastewater threatens not only the aquatic organisms, but also many components of the adjacent ecosystem. One of the major light harvesting pigments in cyanobacteria is phycocyanin which can be rapidly and specifically degraded by external stimuli such as nutritional depletion or environmental stress. We employed the cyanobacterium *Anabaena* sp. PCC 7120 as an indicator organism in estimating the pollution level by phenolic compounds. The phycocyanin content of the cyanobacterium decreased without significantly altering the total chlorophyll as the phenol concentration in a medium increased. We examined the phenol contamination level using the correlation of the phycocyanin content and the phenol concentration. Our results indicated that no significant pollution by phenolic compounds was found in several waterbodies in the vicinity of Daegu, South Korea.

Key words : phenol, cyanobacteria, *Anabaena*, pollution, phycocyanin

INTRODUCTION

Phenol is an aromatic compound that is used as raw material for the production of a variety of resins such as phenolic, epoxy, polycarbonate (Kirk-Othmer, 1978). Phenol and its derivatives are commonly found in industrial wastewater from the production of the manufacturing of synthetic chemicals, pesticides, coal conversion, pulp -paper, and oil-refining. As a toxic and potentially carcinogenic chemical, the release of phenol into the environment is of great concern (IARC, 1999).

The deliberate discharge and accidental release of phenol compounds into the aquatic environment have been reported from many industrialized countries. For instance, a phenol outflow at the Georgia Pacific Company in 1981 polluted the Mississippi river, and water supply stopped for 3 days (the tolerance limit was 0.11 ppm). In South Korea, major phenol pollution in the Nakdong River contaminated the drinking water for two million people in the city of Daegu.

The toxicity of industrial wastewater can influence the operational efficiency of existing wastewater treatment facilities and cause them not to meet effluent standards. The conventional approach for controlling harmful chemicals in the aquatic environment is to use a set of physical-chemical and biochemical parameters (Cronin *et al*., 1991; Trevizo and Nirmalakhandan, 1999). The complex nature of many effluents limits a complete assessment by chemical analysis. How-

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ever, the toxic effects of complex mixtures on wastewater can be detected mostly by biological tests (Chen *et al*., 1999). Throughout the world, where industrial effluent and hazardous waste are growing problems, biological toxicity testing has become one of the most important tools in assessing harmful chemical activity owing to prompt response of living material to the total effect of actual and potential disruptions.

Cyanobacteria are a cosmopolitan group of photosynthetic prokaryotes that play an essential role in aquatic microbial communities. Phycobilisomes (PBS) are the most abundant soluble protein complexes and the major light-harvesting antennae for photosynthesis in cyanobacteria and red algae (Glazer *et al*., 1988; Glauser *et al*., 1992). Phycobiliproteins which carry covalently linked open-chain tetrapyrrole chromophores, bilins, are the primary constituents of phycobilisomes. On the basis of their visible absorption properties, phycobiliproteins have been assigned to four classes: phycoerythrocyanins, phycoerythrins, phycocyanins, and allophycocyanins. Especially, phycocyanin can be rapidly and specifically degraded by external stimuli such as nutritional depletion or environmental stress (Elmorjani *et al*., 1986; Collier and Groossman, 1992; Richaud *et al*., 2001). Since cyanobacteria are found in almost every conceivable habitat, from oceans to fresh water, the phycocyanin content of the cyanobacterium is a good candidate for an indicator organism in estimating the pollution level by phenolic compounds in aquatic environment.

The current study evaluated the phycocyanin content as a bioindicator for tracing phenol pollution, and examined the phenol contamination level using the correlation of the phycocyanin content and the phenol concentration in the Nak-dong River.

MATERIALS AND METHODS

1. Culture conditions and sample collections

Anabaena sp. PCC 7120 was grown in 100 mL BG-11 liquid media with shaking at 30℃ under 100 to 120 μE m^{-2} s^{-2} of illumination. 100 μL of stationary phase culture was used as an inoculum to start the subcultures. Then, different concentrations of phenol ranging from 5 to 200 μg mL^{-1} (5, 10, 20, 25, 30, 50, 100, 150, and 200) were added to the cultures, and incubated for 8 days. A 2 mL sample was taken everyday to measure the growth and phycocyanin content.

The *Anabaena* sp. samples from rivers and lakes were taken from a total of eight places distributed in the northern part of the Nak-dong River including the Kum-ho River, Yul-ha stream, Shin-chon headstream, Young-chon headstream, Gong-san dam, Un-mon dam, An-gye dam, and Young-chon dam in the vicinity of the city of Daegu, South Korea.

2. Measurement of the phycocyanin content

Aliquots of each sample treated with the different concentration of phenol were measured on Shimadzu UV-2401PC spectrophotometer ranging in 620 nm and 750 nm at 25℃. After heating the samples for the degradation of phycocyanin at 75℃, the measurement was repeated. A750 was used as a correction for cell scattering. The phycobilisome content was estimated by relying on quantification of phycocyanin, since virtually all of the phycocyanin was assembled into phycobilisomes. The loss of phycobiliprotein absorbance in samples of a culture heated at 75℃ for 8 min (Bryant, 1996) was used to determine phycocyanin content according to the following equation (Collier and Grossman, 1992).

$$\text{phycocyanin mL}^{-1} = [A620 - A750\,(\text{unheated})] - [A620 - A750\,(\text{heated})]$$

RESULTS AND DISCUSSION

To evaluate whether the phycocyanin content of *Anabaena* sp. PCC 7120 can be used as a bioindicatior, the growth and phycocyanin contents were measured photometrically after different amounts of phenol were added to the culture media. Cyanobacterial cells were grown in BG-11 liquid media and used as inoculums to start subcultures containing various amounts of phenol ranging from 0 to 200 μg mL^{-1} (5, 10, 20, 25, 30, 50, 100, 150, and 200 μg mL^{-1}). Despite the relatively high concentration of phenol, *Anabaena* cultures grew without a significant difference up to 200 μg mL^{-1} of phenol addition (Fig. 1). However, the phycocyanin contents showed apparent dissimilarity depending on the phenol concentration. The phycocyanin content was measured (as indicated in the Materials and Methods) every

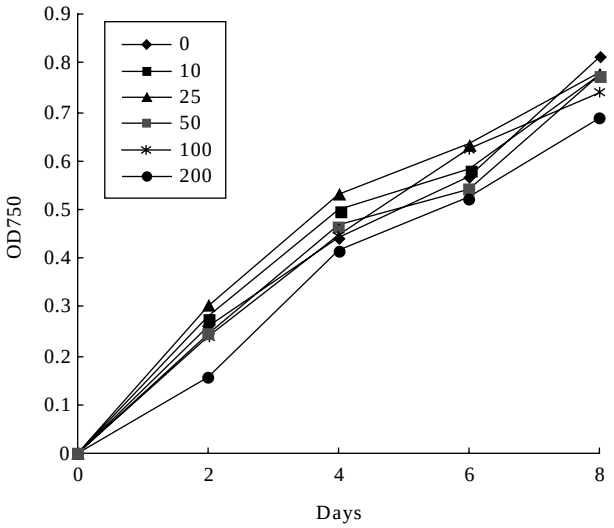

Fig. 1. The growth of *Anabaena* sp. PCC7120 in different concentrations of phenol addition. An isogenic culture of *Anabaena* was grown in BG-11 media until stationary phase and used as an inoculum. Different concentration of phenol (0-200 μg mL^{-1}) was added into the media at the beginning of the subculture. Data are mean values of quadruplicates.

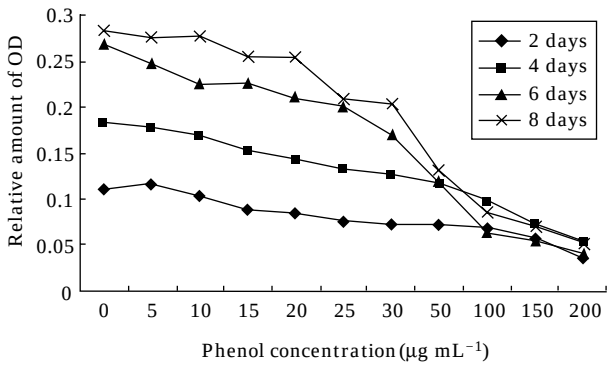

Fig. 2. The relationship between the relative amount of OD and phenol concentration. Each symbol indicates the change of phycocyanin content depending on the different concentration of phenol treatment. The range of phenol concentration was from 5 μg to 200 μg mL^{-1}. The relative amount of OD was measured for tracing the effect of phenol for eight days. One representative result from four independent experiments are shown.

other day and showed a gradual decrease in higher phenol concentrations (Fig. 2). The phycocyanin content expressed as relative amount of OD decreased gradually in the range of 5-30 μg

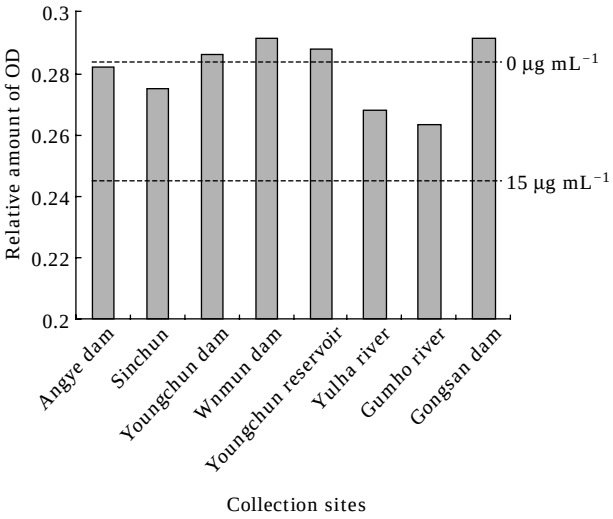

Fig. 3. Estimation of phenol contamination in the Nak-dong River by phycocyanin content of the collected samples containing *Anabaena* sp. The samples were taken from a total of eight places distributed in the northern part of the Nak-dong River. The relative amount of OD in the samples taken from these places ranged from 0.26 to 0.29. The dotted lines indicate the phycocyanin content of cells treated with the phenols ranging from 0 to 15 μg mL^{-1}.

mL^{-1} to the proportion of phenol concentrations, and showed a precipitous decline from 30 to 200 μg mL^{-1}. Our results are consistent with the previous studies in that the phycocyanin content of cyanobacteria was diminished by the environmental stresses without significantly altering the total chlorophyll. Therefore, we concluded that the phycocyanin content of cyanobacteria can be used as a bioindicator to detect the phenol pollution in aquatic environments.

We estimated the phenol contamination level of the water samples collected from the Nak-dong River using the phycocyanin content of cyanobacteria residing therein. The phenol concentrations estimated by phycocyanin content ranged from 0.26 at the Gum-ho River to 0.29 at Gongsam dam (mean value=0.28) (Fig. 3). Figure 3 shows that the values of the samples taken from eight places of the Nak-dong River were close to those of the phycocyanin content of *Anabaena* culture with no phenol treatment (measurement at Fig. 2). Phycocyanin contents of *Anabaena* sp. from all water samples were within the range of 0 to 15 μg mL^{-1} in line with the

estimation of our earlier experiments (Fig. 2). Taking the experimental variations into consideration, it is possible to conclude that phenolic contamination in our water samples were close to undetectable. Several waterbodies in the vicinity of Daegu did not seem to be contaminated by phenolic compounds at the times of our sampling.

Monitoring water pollution is an important task. Several studies have indicated that biological tests with plants, mosses, and cyanobaceria are useful for detecting pollution level in the environment. Pardos *et al.* (1998) demonstrated that the short-term algae *S. capricornutum* biotest based on ^{14}C-fixation is useful to measure the impact of single toxicants of compounds released by suspended sediments on aquatic plants. The current results showed that the phycocyanin content of *Anabaena* sp. can be a useful tool for monitoring phenol contamination in aquatic environments. Further study is needed for more precise estimation of phycocyanin response to various environmental pollutants containing phenolic compounds.

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