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# Variation with N : P ratio of medium and growth stage of microcystin content in *Microcystis aeruginosa*

Min-Ho Jang*[1], Seog June Lee, Byung-Dae Yoon and Hee-Mock Oh
Environmental Microbiology Research Unit, Korea Research Institute of Bioscience and Biotechnology, [1]Dept. of Biology, Pusan National University

## 1. Introduction

Blooms of cyanobacteria frequently occur in eutrophic lakes and polluted water[1]. Strains of the genera, *Microcystis*, *Anabaena*, *Oscillatoria*, and *Nodularia*, which produce various hepatotoxic peptide toxins[2], are often involved. In bodies of water, the most recognized causative agents for cyanobacterial mass occurrences are eutrophication, warm water temperatures, high light intensity, and stable water condition[3]. Surveys of these water blooms have shown that 25 to 95% of them are toxic[4]. However, it is poorly understood what causes a water bloom to be toxic, or how the toxin content of a single strain varies. *Microcystis* strains coexisting in the water blooms are known either to produce hepatotoxins or to be nontoxic[5], and microcystins produced by these strains show a high degree of structural variation[6].

The study on the change of microcystin content in *Microcystis* has been rare published. We investigated content in and relative proportions of microcystins in *M. aeruginosa* and cellular carbon, nitrogen, phosphorus and protein under several N : P ratios.

## 2. Materials and Methods

*Microcystis aeruginosa*, UTEX 2386, was obtained from UTEX, USA., in 1997. This strain has been shown to produce three different microcystin variants. To study the effect of phosphorus and nitrogen on growth, toxin levels, and relative amounts microcystins in their growth media, two experiments were conducted in batch cultures in 1000 ㎖ flasks with 300 ㎖ of growth medium (Table 1).

Table 1. Variation of N : P ratio in the growth medium

| Sample No. | N : P | N-fixed | | P-fixed | |
|---|---|---|---|---|---|
| | | N-$\mu$㏖ · $L^{-1}$ | P-$\mu$㏖ · $L^{-1}$ | N-$\mu$㏖ · $L^{-1}$ | P-$\mu$㏖ · $L^{-1}$ |
| 1 | 1 : 1 | 71.40 | 71.40 | 6.50 | 6.50 |
| 2 | 5 : 1 | 71.40 | 14.28 | 32.50 | 6.50 |
| 3 | 16 : 1 | 71.40 | 4.46 | 104.00 | 6.50 |
| 4 | 50 : 1 | 71.40 | 1.43 | 325.00 | 6.50 |
| 5 | 100 : 1 | 71.40 | 0.71 | 650.00 | 6.50 |

The growth medium was SW media[7]. In the nutrient medium experiments, the

concentration of phosphorus and nitrogen, were regulated by amount of $K_2HPO_4$ and $NaNO_3$. The flasks were incubated with continuous shaking, constant temperature 30℃, and continuous cool white fluorescent irradiance. Growth was followed by measuring of dry weights at each sampling day by filtering 10 mℓ of the culture to tared GF/C, which were then dried for 24 h. During the incubation, fluorescence and $OD_{680}$ were measured nearly ever day. Content of total carbon, nitrogen, phosphorus, and protein in algal cell were measured. For toxin analysis, the cells were harvested by centrifugation at 10,000 rpm at 4℃, frozen, and stored at -70℃ until analyzed. From the freeze-dried cell material, the toxins were extracted two times with 10 mℓ 5% acetic acid for 30 min while stirring. The extract was centrifuged at 3500 rpm and then the supernatant was applied to a $C_{18}$ cartridge. Microcystins were finally eluted from the $C_{18}$ cartridge with 10 mℓ of methanol. The eluate was evaporated under reduced pressure and then the residue was dissolved in 1 mℓ of methanol. The solution was subjected to HPLC analysis (238 nm, pH 3.0)[8]. The concentrations of the toxins were determined by extrapolating peak areas to a calibration curve determined with purified MC-LR, -RR, and -YR as a standard. The detection limit for the microcystins in cell material was 0.1 μg/g.

3. Results and Discussion

During the incubation at N and P fixed, fluorescence and $OD_{680}$ were measured as growth rate of *M. aeruginosa*. As fixed nitrogen and different condition of phosphorus, fluorescence was increased at 3 days after incubation (sample 4, 137±3), but sample 5 (lowest phosphorus) was lowest (99±3). $OD_{680}$ was gradually increased no effect of phosphorus concentrations after incubation. The fluorescence of fixed phosphorus, sample 4 (161±1), lower nitrogen than sample 5, was more higher. The highest value of fluorescence was arrived at 6-7 days after incubation. Sample 1 (118 ±5) and 2 (127±6) were maximum peak at 6 days. $OD_{680}$ of fixed phosphorus was no significant effect on fixed nitrogen.

The case of nitrogen fixed condition, cell dry weight had no significant effect on phosphorus concentration, but chlorophyll-a increased in the cells grown at high phosphorus concentrations. Under the phosphorus fixed condition, cell dry weight and chlorophyll-a were increased in the cells grown at high phosphorus concentrations. In the nitrogen and phosphorus fixed experiments, no significant effect was seen in the growth but these results were similar in the results of Watanabe and Oishi[3].

The case of nitrogen fixed condition, carbon and nitrogen in the cell had no significant effect on phosphorus concentration, but sample 1, grown at high phosphorus concentrations, was measured maximum phosphorus (1.47±0.03) and sample 5 was measured minimum phosphorus (0.78±0.05), grown at low phosphorus concentrations. Protein, under the nitrogen fixed condition, were a little increased in the cells grown at high phosphorus concentrations. Protein in the cell grown at phosphorus fixed condition had no significant effect on nitrogen concentration.

In the experiments of phosphorus and nitrogen fixed, toxin of *M. aeruginosa* differed from nitrogen and phosphorus concentration (Table 2).

Table 2. Comparison of microcystin LR, RR, and YR in algal cell

| Sample | N-Fixed condition (μg/g-cell) | | | P-Fixed condition (μg/g-cell) | | |
|---|---|---|---|---|---|---|
| No. | MC-LR | MC-RR | MC-YR | MC-LR | MC-RR | MC-YR |
| 1 | 293.0±60.4 | 1393.9±30.6 | 97.0±37.8 | 233.5±13.1 | 1722.7± 59.4 | 84.0±37.3 |
| 2 | 212.0± 2.4 | 1293.6±31.3 | 93.9±30.1 | 255.9±39.7 | 1740.4±160.5 | 62.3±29.8 |
| 3 | 250.4±11.5 | 1545.6± 9.3 | 100.0±34.2 | 348.4±37.1 | 2273.0±174.2 | 116.9±20.7 |
| 4 | 218.0± 8.5 | 1288.6±44.4 | 81.2±24.2 | 370.3± 9.6 | 2172.2±291.7 | 123.4±43.9 |
| 5 | 318.6± 6.3 | 1586.7±80.2 | 86.7±15.8 | 248.0±24.6 | 1557.5±130.8 | 94.5±59.3 |

Under the nitrogen fixed condition, sample 5 was highest detected total microcystins and MC-LR, -RR were did. MC-YR was highest detected to sample 3 (P=4.46 μmol/L). However, all of nitrogen fixed sample's toxin had no significant effect on the phosphorus concentrations. In the phosphorus fixed condition, MC-RR was highest detected to sample 3 and MC-LR, -YR were more detected to sample 4. As the nitrogen and phosphorus fixed condition, 16 : 1 ratio of nitrogen and phosphorus was measured high toxin amounts.

In these results of nitrogen and phosphorus fixed experiments, the most amounts of microcystin was MC-RR. But in the results of Rapala *et al*[9], MC-LR was shown more amounts than MC-RR due to difference of species.

When nitrogen to phosphorus ratio was 16 to 1, cell growth and toxin amount were measured. Chlorophyll-a increased to 4 days after incubation but gradually decreased after 4 days. Total carbon also was shown similarly pattern to chlorophyll-a. Total nitrogen gradually decreased from incubation but dramatically decreased at 4 days. Total phosphorus also decreased.

Toxin amounts was shown similarly pattern to chlorophyll-a. Toxin was detected maximum amount at 4 days (2190.7±727.6) and slowly decreased. MC-LR and -RR were detected maximum amount at 4 days (508.2±19.6, 1543.0±111.3 μg/g-cell) but MC-YR decreased (139.5±3.8 μg/g-cell). In the result, MC-LR, -RR, and -YR amounts in the cell changed in the cell growth. But in the results of Rapala *et al*[9], microcystins amounts were variable changed in the each species and each incubating times. In their results, microcystins were detected maximum amounts at 10 and 15 days.

4. Abstract

The change of microcystin content in *Microcystis aeruginosa* UTEX 2388 was investigated at several N : P ratios of culture medium and along the growth stages. The medium N : P ratios were set to 1:1, 5:1, 16:1, 50:1 and 100:1 under both N- and P-fixed condition. Microcystin content in the N-fixed was not changed much, whereas that in the P-fixed was the highest value of 2747.6 ±180.3 μg/g-cell at a N : P ratio of 16:1 after 8 days incubation. Microcystin-RR (MC-RR) was more than

the other microcystins (MC-LR and -RR) at all examined samples. When the N : P ratio of medium was fixed to 16:1, microcystin content which expressed per cell dry weight was highest at 2190.7±115.3 $\mu g/g$-cell after 4 days incubation and then gradually decreased. Cellular carbon and chlorophyll-*a* contents of algal culture were also highest at day 4 and also showed a similar decline. From these results, it is believed that there is a clear relationship between microcystin content in *M. aeruginosa* UTEX 2388 and cellular carbon or chlorophyll-*a* content.

5. Reference


Lindholm, T., J. E. Eriksson, and M. Reinikainen. 1992. Ecological effects of hepatotoxic cyanobacteria. Environ. Toxico. Water Qual.. 7 : 87-93.

Skulberg, O. M., G. A. Codd, and W. W. Carmichael. 1984. Toxic blue-green algal blooms in Europe: A growing problem. Ambio. 13 : 244-247.

Watanabe, M. F., and S. Oishi. 1985. Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. Appl. Environ. Microbiol. 49 : 1342-1344.

Baker, P. D., and A. R. Humpage. 1994. Toxicity associated with commonly occurring cyanobacteria in surface water of the Murray-Darling basin, Australia. Aust J. Mar. Freshwater Res. 45 : 773-786.

Sivonen, K., S. I. Niemelä, R. M. Niemi, L. Lepistö, T. H. Luoma, and L. A. Räsänen. 1990. Toxic cyanobacteria (blue-green algae) in Finnish fresh and costal waters. Hydrobiologia, 190 : 267-275.

Sivonen, K., M. Namikoshi, R. Luukkainen, M. Färdig, L. Rouhiainen, W. R. Evans, W. W. Carmichael, K. L. Rinehart, and S. I. Niemelä. 1995. Variation of cyanobacterial hepatotoxins in Finland. P. 163-169. *In* M. Munawar and M. Luotola (ed.), The contaminants in the Nordic ecosystem : dynamics, processes and fate. Ecovision World Monograph Series. SPB Academic publishing, Amsterdam, The Netherlands.

Smith and Wiedeman. 1964. Canadian J. Botany. 42 :

Harada, K. I., K. Matsuura, M. Suzuki, H. Oka, M. F. Watanabe, S. Oishi, A. M. Dahlem, A. R. Beasley, and W. W. Carmichael. 1988. Analysis and purification of toxic peptides from cyanobacteria by reversed-phase high-performance liquid chromatography. J. Chromatogr. 448 : 275-283.

Rapala, J., K. Sivonen, C. Lyra, and S. I. Niemelä. 1997. Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a fuction of growth stimuli. App. Environ. Microbiol. 63 : 2206-2212.