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Toxin Production by *Microcystis aeruginosa* as a Function of Light in Continuous Cultures and Its Ecological Significance

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The effects of light intensity and light quality on toxin production by *Microcystis aeruginosa* were examined in continuous cultures. Light intensity had a pronounced effect on toxicity and the toxin production rate. Toxicity and the toxin production rate increased with light intensity up to an intensity of about 40 microeinsteins m^{-2} s^{-1} and decreased at higher light intensities, while the ratio of toxin to protein was constant at intensities of more than 40 microeinsteins m^{-2} s^{-1}. Light quality had only slight effects on toxicity. The results of our laboratory experiments were supported by the results of field work in which we examined toxin production at different depths in a lake. Our observations explain the mixed pattern of high and low toxicity found in a surface bloom of *M. aeruginosa*. Our findings also indicate that production of the peptide toxin can be uncoupled from general protein synthesis.

It has been known since 1964 that the possibility that a cyanobacterial bloom may be toxic is more than 50% (14). It has also been recognized for some time that cyanobacterial toxicity may vary weekly at the same sampling point (4). Mixed patterns of high- and low-toxicity areas in a lake have also been found to be common (3, 6, 8). An investigation over a 3-year period (1984 to 1986) (2) showed that each year *Microcystis aeruginosa* was nontoxic at the beginning of the growing season and developed high toxicity during the first strong biomass increase. In addition, toxicity has been found to vary not only for different strains but also for different clones of the same isolate (3). It is still not known why some cyanobacteria belonging to the same species produce toxins whereas others do not, and it is not known whether there are environmental factors which convert an organism that does not produce toxin into a toxin producer. The mechanism behind the differences in toxicity is not understood and must be clarified before predictions on the pattern of toxin production can be made. This kind of information, together with toxicological data, is a prerequisite for having informed discussions on health risk assessments.

It has been suggested (19) that toxin production may be predictable if working models that compare nutrient and environmental conditions with bloom dynamics can be developed. However, more research on environmental factors that promote toxin production is needed (16). The studies described below were conducted by using continuous cultures. This is a cultivation system which allows workers to study the effect of one factor at a time, in contrast to batch cultures. Our study was based on factors that have been implicated previously in the toxicity of hepatotoxin-containing cyanobacteria in batch cultures (20, 23).

MATERIALS AND METHODS

Organism and growth conditions. The organism which we used was *Microcystis aeruginosa* CYA 228/1, which was obtained from the Norwegian Institute for Water Research. This organism was grown axenically in continuous cultures and in the medium described by Van Liere and Mur (21). Continuous illumination was provided by circular Philips type TLEM 40 W/33 white fluorescent lamps. The incident irradiance was measured outside the flasks with a Li-cor quantum photometer (model Li-188B). The culture was aerated with ambient air that was filtered through activated carbon and sterilized cotton; the flow rate was 72 liters h^{-1}. The temperature was maintained at 20°C.

Different light qualities were obtained by wrapping the culture vessels in nonmonochromatic plastic foils. The red filter absorbed wavelengths between 440 and 600 nm, with a peak at 556 nm, and the green filter absorbed wavelengths between 300 and 500 nm and between 550 and 700 nm, with peaks at 433 and 635 nm.

Analytical methods. Dry weights were determined by filtering 1- to 10-ml portions of cultures through preweighed cellulose nitrate filters (pore size, 0.8 μm; Sartorius). The filters were dried to constant weight at room temperature in a silica gel desiccator.

Samples (1 ml) for protein determinations were collected in acid-washed glass tubes and stored at −20°C until the experiment was completed. Prior to assay the samples were freeze-dried. Protein contents were estimated by using the Lowry method (10). Standard solutions of bovine serum albumin were used for calibration purposes.

Toxin determination. Samples (50 to 300 ml) were collected and frozen at −20°C. After thawing, glacial acetic acid was added to a final concentration of 5%. The samples were then extracted for 90 min with stirring at 4°C. After centrifugation (at 9,000 rpm for 10 min in a Sorvall type RC-5B Superspeed apparatus), the pellets were reextracted with 5% acetic acid, and the pooled supernatants were applied to activated C_{18} cartridges. The cartridges were rinsed with water and eluted with 5 to 10 ml of methanol. The toxin content was quantified by using high-performance liquid chromatography performed with an internal surface reverse-phase column (Regis) (13). The mobile phase was 12% acetonitrile–88% 0.1 M KH_2PO_4 (pH 6.8), and the flow rate was 1 ml/h. The detector was set at 238 nm. All peaks were tested for toxin by using a standard mouse bioassay (15). The toxin was quantified by using purified toxin standards obtained for *M. aeruginosa*.

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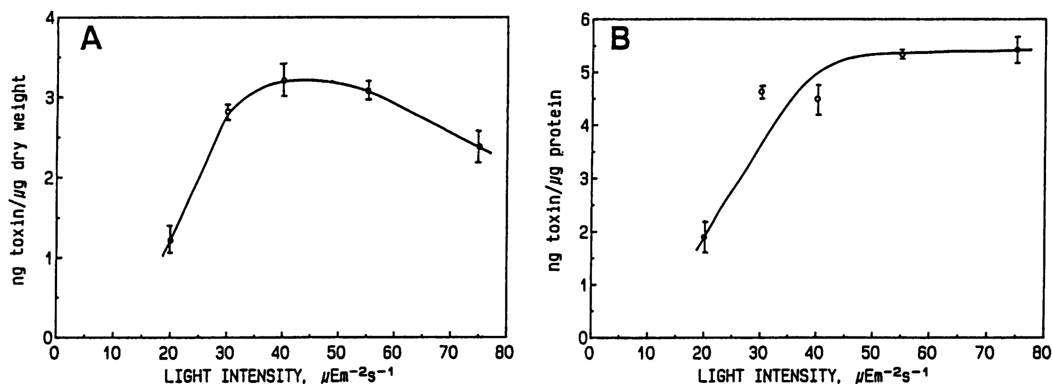

FIG. 1. Effect of light intensity on toxicity (A) and the ratio of toxin to protein for *M. aeruginosa* grown in a continuous culture at a *D* of 0.013 h^{-1}. μE, microeinsteins.

RESULTS

Light intensity. The effect of increasing the light intensity on the steady-state dry weight of the cyanobacterium *M. aeruginosa* grown in continuous cultures at a growth rate (*D*) of 0.013 h^{-1} revealed that light was the limiting growth factor at light intensities less than 75 microeinsteins m^{-2} s^{-1}. An increase in light intensity from 20 to 75 microeinsteins m^{-2} s^{-1} resulted in an increase in culture toxin content from 300 to 2,700 ng/ml.

The toxicity of the organism was determined by calculating the ratio of toxin to biomass and was found to increase about 2.5-fold when the light intensity was increased from 20 to 40 microeinsteins m^{-2} s^{-1} (Fig. 1A). A further increase in light intensity resulted in a decrease in toxicity. The toxin produced by *M. aeruginosa* is a small peptide (12) and consequently is part of the total cellular protein. We also found that the ratio of toxin to protein increased with light intensity up to 40 microeinsteins m^{-2} s^{-1}, but in contrast to toxicity this relationship was almost unaffected by any further increase in light intensity (Fig. 1B). The decrease in toxicity at light intensities of more than 40 microeinsteins m^{-2} s^{-1} (Fig. 1A) may have been associated with accumulation of carbohydrates at high light intensities. The increase in the ratio of toxin to protein with light intensity indicated that toxin synthesis increased faster than general protein synthesis at light intensities between 20 and 40 microeinsteins m^{-2} s^{-1}.

Light quality. Since light intensity appeared to have an effect on toxicity and the ratio of toxin to protein, we examined whether light quality affected these relationships. Therefore, *M. aeruginosa* was grown under white light, green light, and red light at intensities of 20 microeinsteins m^{-2} s^{-1} and a *D* of 0.013 h^{-1}. Table 1 shows that both toxicity and the ratio of toxin to protein were slightly enhanced by both red light and green light compared with white light. This response must be investigated further, since the filters which we used were not monochromatic.

TABLE 1. Effect of light quality on toxicity and the ratio of toxin to protein for *M. aeruginosa* grown in continuous cultures[a]

Light quality	Toxicity (ng of toxin per μg [dry wt])	Ratio of toxin to protein[b]
Red	1.84 ± 0.40	2.60 ± 0.87
White	1.17 ± 0.15	1.93 ± 0.38
Green	1.58 ± 0.21	2.39 ± 0.21

[a] The *D* was 0.013 h^{-1}, and the light intensity was 20 microeinsteins m^{-2} s^{-1}.

[b] Nanograms per microgram.

In order to examine how fast the toxicity of *M. aeruginosa* can be altered by changes in light intensity, continuous cultures at a *D* of 0.013 h^{-1} were subjected to changes in light intensity. Figure 2 shows that a change in light intensity from 45 to 10 microeinsteins m^{-2} s^{-1} resulted in a decrease in toxicity about 4 to 5 days after the change, while it took somewhat longer for toxicity to increase after a change from 12 to 37 microeinsteins m^{-2} s^{-1}. The patterns shown in Fig. 2 (toxicity first increased after light intensity was decreased or decreased after light intensity was increased) were obtained every time that the experiments were performed.

Toxin production by *M. aeruginosa* at different depths was examined during a *M. aeruginosa* bloom in a eutrophic lake, Lake Akersvann in Norway. *M. aeruginosa* suspended in the medium (21) used for the continuous cultures was placed into 250-ml bottles, and the bottles were suspended on strings at depths of 0, 1, 3, and 5 m. The values for toxicity and the ratio of toxin to protein were determined after 7 days. Figure 3 shows that toxicity and the ratio of toxin to protein decreased with depth; these results confirmed the effects of light intensity on toxin production which were observed in continuous cultures.

DISCUSSION

The influence of light intensity on the toxicity of *M. aeruginosa* grown in batch cultures has been studied by Watanabe and Oishi (23), who observed a fourfold increase in the toxicity of this organism when they increased the light intensity from 7.53 to 30.1 microeinsteins m^{-2} s^{-1}. The toxicity was determined by measuring 50% lethal doses in a mouse bioassay. Similar results were obtained by Van der Westhuizen and Eloff (20). In both of these studies, toxicity was independent of light intensity at higher intensities, in contrast to the decrease in toxicity observed in our study. We observed that the ratio of toxin to protein was independent of light intensity at intensities between 40 and 75 microeinsteins m^{-2} s^{-1} (Fig. 1B). Since the effects of alterations in both growth rate and light intensity were studied at the same time by Watanabe and Oishi (23) and Van der Westhuizen and Eloff (20) and since toxicity was determined by using a mouse bioassay, the findings of these authors are not directly comparable with our results. Ac-

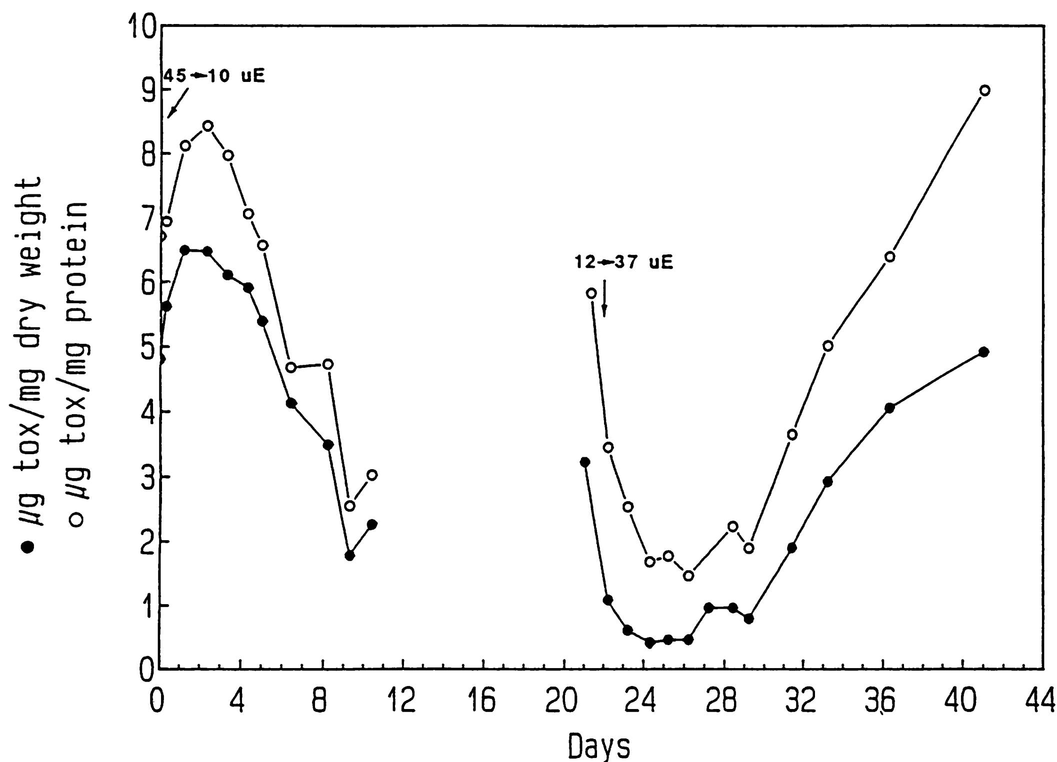

FIG. 2. Effect of changes in light intensity on toxicity and the ratio of toxin to protein for *M. aeruginosa* grown in continuous cultures at a D of 0.013 h^{-1}. Only one set of data is shown, but the experiment was repeated three times and the same pattern was obtained every time. μE, microeinsteins.

cording to Wicks and Thiel (24), Harris and Gorham (9a) reported only small differences in toxicity at light intensities of 37 and 270 microeinsteins m^{-2} s^{-1}. A positive correlation has been found between solar radiation and the toxicity of *M. aeruginosa* under natural conditions (24). On the other hand, Codd and Poon (5) observed no difference in toxicity when they increased the light intensity from 5 to 50 microeinsteins m^{-2} s^{-1} for their *M. aeruginosa* strain. The conflict-

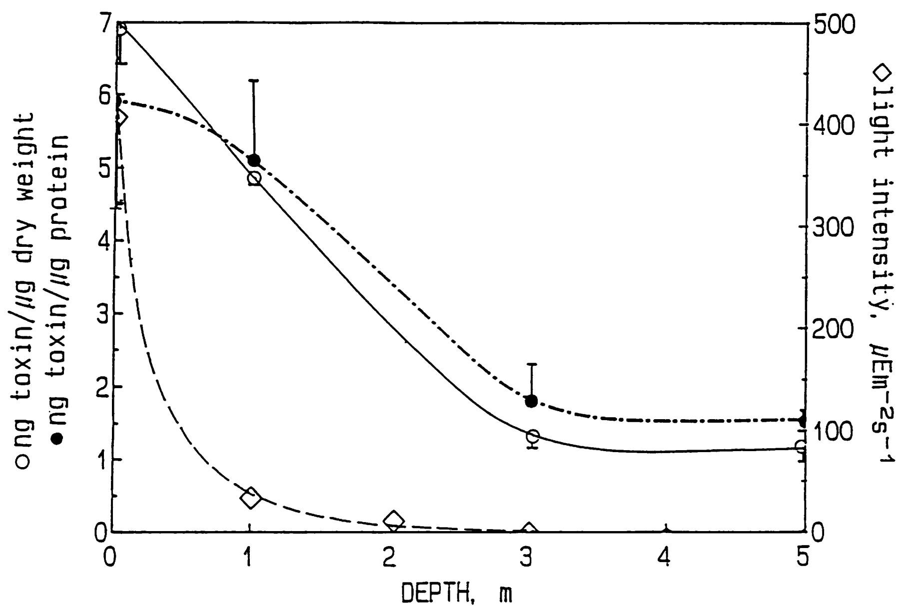

FIG. 3. Toxicity and ratio of toxin to protein for *M. aeruginosa* cultures enclosed in 250-ml bottles at different depths in Lake Akersvann, Norway, during a bloom of *M. aeruginosa*. The light intensities at the different depths are the average values from the day that the experiment was started and the day that the experiment was terminated. μE, microeinsteins.

ing results obtained by Codd and Poon (5) were probably not caused by differences in the light spectrum used, as suggested by Sivonen (18), because Table 1 shows that changes in light quality had minor effects on toxicity. It is more likely that these results were due to different growth conditions; for example, Gorham (9) has shown that the aeration rate influences the toxicity of *M. aeruginosa*.

Results obtained for different strains of *M. aeruginosa* (20, 23, 24; this study) and for other cyanobacteria, such as *Anabaena* and *Aphanizomenon* spp. (7) and an *Oscillatoria* sp. (18), clearly demonstrate that light intensity (available energy) is a controlling factor for toxicity in many cyanobacteria. The light intensities that influence the toxicity of *M. aeruginosa* seem to be less than about 40 microeinsteins m^{-2} s^{-1}. During bloom conditions, such light intensities can be found at a depth of about 1 m (Fig. 3); therefore, the light intensities that have been shown to influence toxicity under laboratory conditions are relevant under natural conditions.

The decrease in toxicity (ratio of toxin to dry weight) at light intensities of more than 40 microeinsteins m^{-2} s^{-1} does not imply that these light intensities elicit toxicity or that surface-dwelling populations can be expected to be nontoxic. The decrease in the ratio of toxin to dry weight at high light intensities (Fig. 1A) is probably caused by an accumulation of polysaccharides, which would increase the dry weight of the organism, since Fig. 1B shows that the ratio of toxin to protein remains constant at light intensities above 40 microeinsteins m^{-2} s^{-1}. Therefore, these findings indicate that *M. aeruginosa* should have maximal toxin content at the surface and that the content should decrease with depth. This hypothesis is supported by the results presented in Fig. 3, which shows that the toxicity of *M. aeruginosa* varied with depth, although incubation of samples in small bottles is not a particularly meaningful field evaluation of the laboratory results. Further support for this conclusion comes from a Swedish investigation in which the levels of toxicity at different depths of a *Microcystis* bloom were determined (1). Annadotter et al. found that the toxicity was highest at the surface and decreased with depth during periods of calm weather, while this was not the case after windy periods, when mixing of water from different depths occurred.

Many planktonic cyanobacteria can regulate their buoyancy and move vertically (11, 17); a rate of 1 m h^{-1} has been observed for *Oscillatoria agardhii* during calm weather (22). An organism can also be transported quickly from one depth to another by currents. These movements, together with the effect of light intensity (depth) on toxicity and the relatively long time that it takes for the organism to change toxicity (Fig. 3), can explain the mixed pattern of high and low levels of toxicity found in surface blooms, since the toxicity of organisms that have been at the surface (high light intensity) for some time would be different from the toxicity of organisms that have just surfaced from greater depths (low light intensity).

The unexpected short-term changes in the ratio of toxin to dry weight or the ratio of toxin to protein when cultures were subjected to different changes in light intensity (Fig. 2) could be explained by dilution or accumulation of an inducer of toxin production. At a light intensity of 45 microeinsteins m^{-2} s^{-1}, toxin production is constant and probably at its maximum (Fig. 1), and if an inducer is involved, its cellular concentration would be high. After a decrease in light intensity, the growth rate at first decreases (dilution of culture), and then it stabilizes again at the same growth rate as before the change. During the period when the growth rate is reduced, the production of toxin is still high because of a high concentration of inducer; however, after some cell divisions the inducer is diluted, and toxin production decreases. When the light intensity is increased, the inducer has to accumulate before the toxin content increases. Experiments to study the possibility that toxin production is controlled by changes in cellular concentrations of an inducer are in progress.

TABLE 2. Effect of light intensity on toxin production and overall protein production by *M. aeruginosa* grown in continuous cultures at a *D* of 0.013 h^{-1}

Light intensity (microeinsteins m^{-2} s^{-1})	Toxin production (ng of toxin per mg per h)	Protein production (μg of protein per mg per h)
20	15.9 ± 1.7	8.19 ± 1.21
30	36.7 ± 0.8	7.93 ± 0.41
40	41.7 ± 2.3	8.94 ± 0.01
55	40.3 ± 1.2	7.50 ± 0.23
75	30.9 ± 2.4	6.12 ± 1.08

In continuous cultures, the rate of production of cells or cellular compounds is equal to the dilution rate, at steady state. Therefore, the rates of toxin production per milligram of cells at different light intensities can be calculated by dividing the toxin content by the dry weight and multiplying the result by the dilution rate. Such calculations showed (Table 2) that the toxin production rate increased about 2.5-fold when the light intensity was increased from 20 to 40 microeinsteins m^{-2} s^{-1}, while the protein production rate was almost constant under these conditions. The maximal toxin production rate which we found was about 40 ng/mg (dry weight) per h.

Our results strongly suggest that light intensity is an important factor in the control of peptide toxin production in *M. aeruginosa* and that changes in this physical factor can uncouple the production of toxin from general protein synthesis. Therefore, alteration of light intensity seems to be an excellent tool for further investigations of the molecular mechanisms behind toxin production.

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