

Supporting Information

Kinetics of *Microcystis aeruginosa* growth and intracellular microcystins release after UV irradiation

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Methods

UV irradiation and subsequent incubation

Axenic cultures of *M. aeruginosa* were grown in BG-11 medium to reach the exponential growth phase. *Microcystis aeruginosa* suspensions were washed three times using the BG-11 medium to remove extracellular microcystins prior to UV exposure. To assess the effects of UV irradiation, 40 ml of each cultured algal suspension were irradiated in a 90-mm-diameter glass petri dish. BG-11 medium was used as the exposure medium, as well as the subsequent incubation medium. Absorption of the medium at 254 nm was 0.09. We used a monochromatic low-pressure UV lamp (15 W × 2, GE/Hitachi, Tokyo, Japan) and a polychromatic medium-pressure UV lamp (330 W × 1, B410MW, Ebara, Tokyo, Japan). The germicidal intensity of the light emitted from each lamp was standardized by determining the irradiance of light with a biosimulator using an F-specific RNA coliphage Qβ. The fluence rate for both LP and MP UV lamps was 0.9 mW cm⁻². Fluence rates were fixed throughout the experiments, and UV fluence was controlled by varying the exposure time from 33 to 2000 seconds. After irradiation, samples were incubated for 14 days in an incubation chamber (25°C, 1500 lux of fluorescent light, 12 h/12 h light/dark cycle) in 100-ml Erlenmeyer flasks. Cultures were maintained at 25°C in an incubation chamber (BITEC-400L, Shimadzu, Kyoto, Japan) under controlled lighting. Fluorescent lamps (FL20SW-B, GE/Hitachi, Tokyo, Japan) were used as a light source with an automated light/dark cycle of 12 h/12 h. Light intensity during the lighting phase was 1500 lux. Experiments were conducted three times from UV irradiation to subsequent incubation and analysis, and the results are the means of those three experiments. Error bars in the figures show the maximum and minimum of each data set.

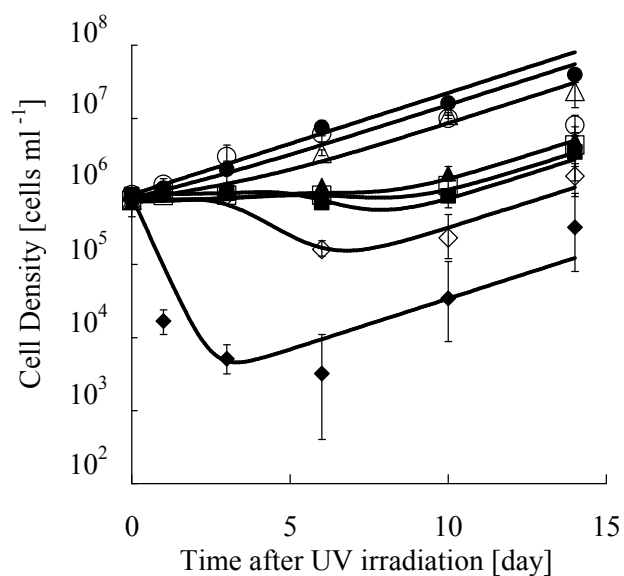
Cell counting

For cell counting, any cells exhibiting chlorophyll fluorescence were counted; cells that may have been inactivated by UV were counted unless they had lost their chlorophyll fluorescence. The numbers of cells were calculated immediately before and after UV irradiation, as well as 1, 3, 6, 10, and 14 days (24 ± 2 h) of incubation after UV irradiation. The numbers of cells are expressed as cell density in the figures; the results presented are averages of ten microscopic fields.

Microcystins analysis

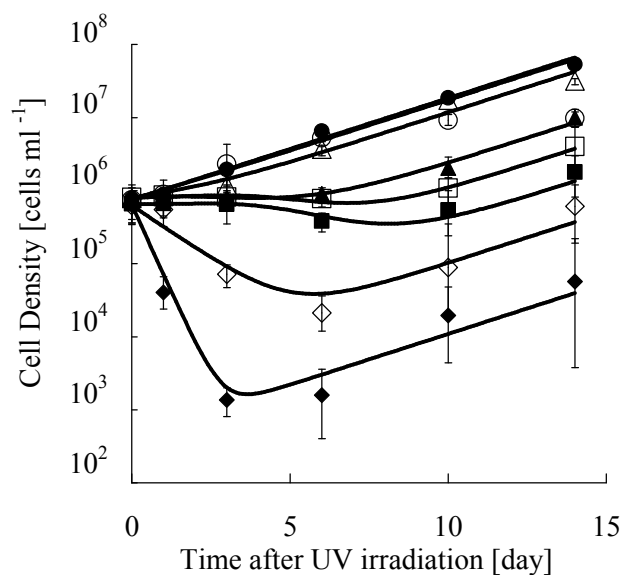
The concentration in each sample was determined by averaging the results from two wells in the 96-well plates. This kit had a detection limit of 50 ng l⁻¹ as Microcystin-LR equivalents. Because the PCC 7806 strain produces Microcystin-LR and the demethylated variants, the results of the ELISA measurement can be considered the concentration of them. To determine the extracellular concentration, each sample was filtered using a 0.45-µm polytetrafluoroethylene membrane and subjected to ELISA. To determine total concentration, each sample was freeze-thawed and then filtered prior to ELISA. To determine intracellular concentration, extracellular concentration was subtracted from the total concentration.

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3 Figure SI1. Results of the cell number model. The symbols indicate the measured cell numbers after 0 (
 4 ○), 30 (●), 60 (△), 90 (▲), 120 (□), 180 (■), 600 (◇), and 1800 mJ cm⁻² (◆) of LP UV. The lines
 5 show the modeled results.



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7 Figure SI2. Results of the cell number model. The symbols indicate the measured cell numbers after 0 (
 8 ○), 30 (●), 60 (△), 90 (▲), 120 (□), 180 (■), 600 (◇), and 1800 mJ cm⁻² (◆) of MP UV. The lines
 9 show the modeled results.

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2 Table SI1. Calculated parameters for LP UV samples.

UV fluence (mJ cm ⁻²)	0	30	60	90	120	180	600	1800
μ (d ⁻¹)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
X_0 (cells ml ⁻¹)	9.11×10^5	8.10×10^5	7.66×10^5	7.24×10^5	7.52×10^5	9.06×10^5	7.75×10^5	8.49×10^5
G_0 (cells ml ⁻¹)	9.11×10^5	6.23×10^5	3.49×10^5	5.66×10^4	3.85×10^4	3.13×10^4	1.29×10^4	1.39×10^3
D_0 (cells ml ⁻¹)	0	1.87×10^5	4.17×10^5	6.67×10^5	7.14×10^5	8.75×10^5	7.62×10^5	8.47×10^5

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4 Table SI2. Calculated parameters for MP UV samples.

UV fluence (mJ cm ⁻²)	0	30	60	90	120	180	600	1800
μ (d ⁻¹)	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
X_0 (cells ml ⁻¹)	7.53×10^5	7.71×10^5	7.89×10^5	7.97×10^5	8.15×10^5	6.55×10^5	6.25×10^5	6.39×10^5
G_0 (cells ml ⁻¹)	7.53×10^5	7.12×10^5	4.76×10^5	9.60×10^4	4.21×10^4	1.51×10^4	4.14×10^3	4.44×10^2
D_0 (cells ml ⁻¹)	0	5.9×10^4	3.12×10^5	7.01×10^5	7.73×10^5	6.40×10^5	6.21×10^5	6.39×10^5

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