Supplementary Materials: Microcystin-LR Biodegradation by *Bacillus* sp.: Reaction Rates and Possible Genes Involved in the Degradation

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1. *M. aeruginosa PCC7820* Culture

Algae were cultured in ASM medium. 150 mL flasks containing the *M. aeruginosa PCC7820* culture were incubated at 25 °C in a 12 h/12 h' light (19.8 μmol·m^{-2}/s intensity) and dark cycles for 55 days. ASM was prepared following the composition described by Ronald M. Atlas [1].

2. *M. aeruginosa PCC7820* Cells Counts

M. aeruginosa PCC7820 cells were enumerated via a hemocytometer (Fuchs-Rosenthal, Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany) under an optical microscope (Microphot-FXA, Nikon, Japan) at a magnification of 100×.

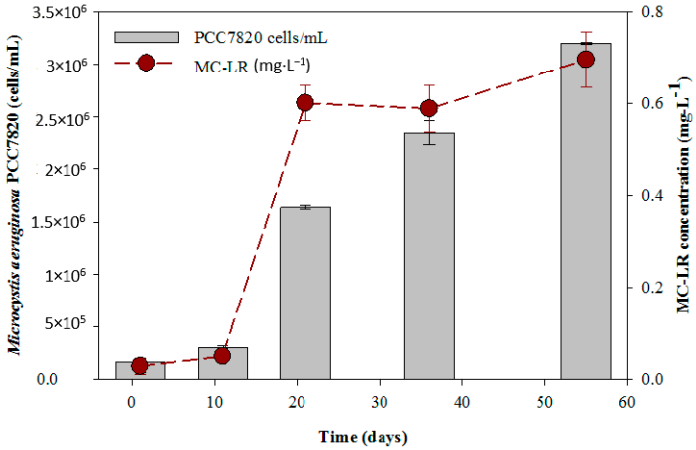


Figure S1. Growth curve of *M. aeruginosa* and the change of MC-LR concentration. (Error bars in the figure represent one standard deviation for three measurements). The cyanobacteria are harvested during the period with a high amount of MC-LR. For the current study, the cyanobacteria were taken at the 55th day of the culture and had a MC-LR concentration of 10 mg·L^{-1} (15 mL).

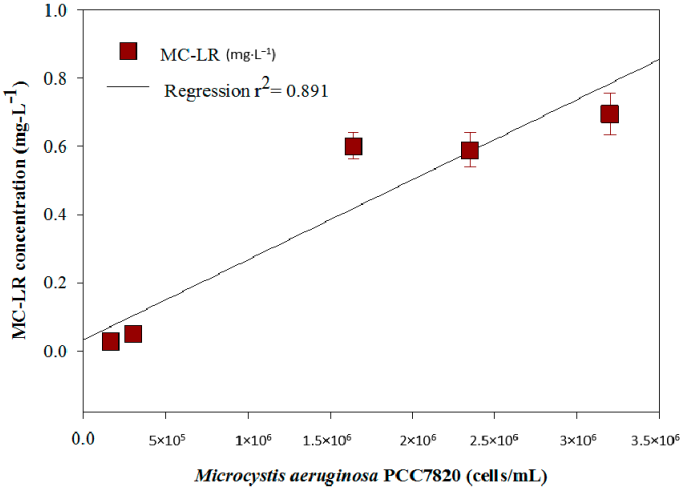


Figure S2. Correlation between MC-LR (mg·L^{-1}) and *M. aeruginosa PCC7820* cells/mL. (Error bars in the figure represent one standard deviation for three measurements). The intercept is 0.03 and the slope of the linearity is 0.02 mg·L^{-1}.

3. *mlr*A Gene Standard Curve

The standard curve for *mlr*A gene abundance and CT values.

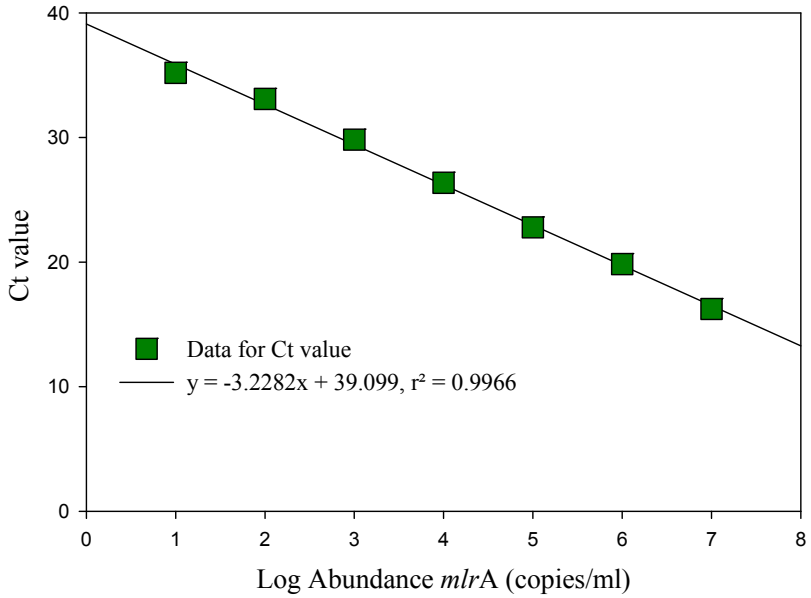


Figure S3. *mlr*A gene standard curve.

4. Total Bacteria Gene Standard Curve

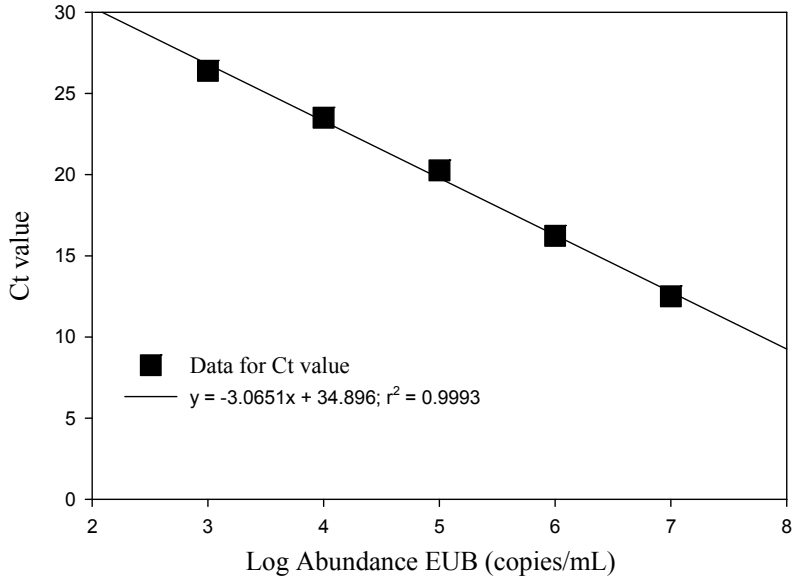


Figure S4. EUB gene standard curve for total bacteria.

5. MC-LR Measurement Standard Curve Using ELISA

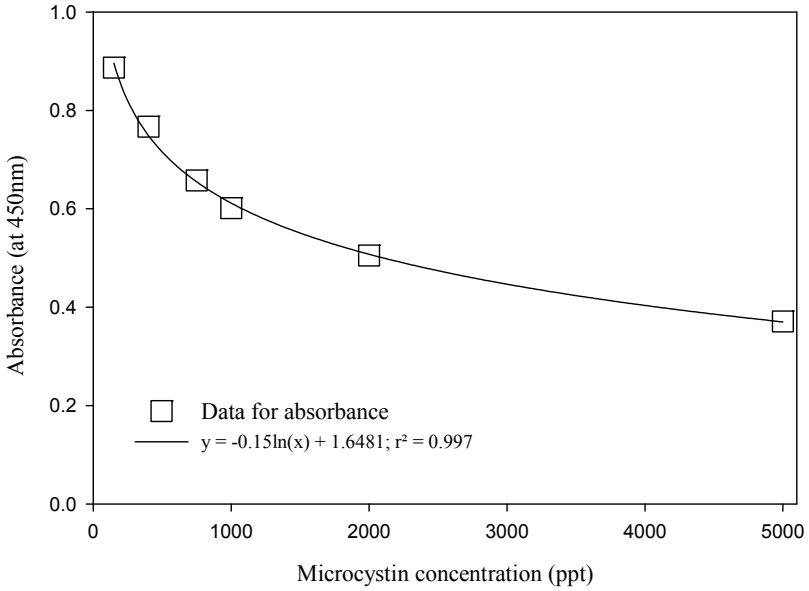


Figure S5. MC-LR measurement standard curve via ELISA.

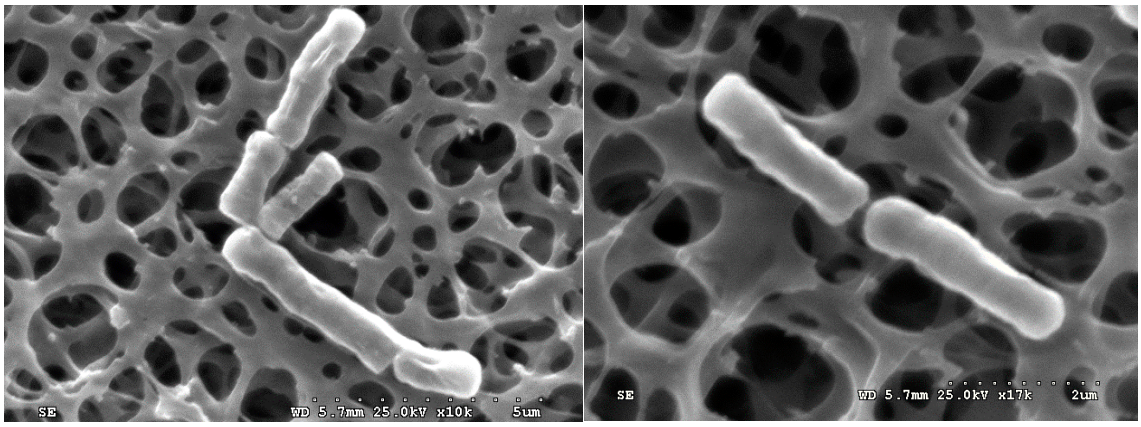
6. Bacterial Growth Culture Solutions

Mineral salt medium (MSM): composition of the MSM used was as follows (g/L): $NaNO_3$ (2.0 g/L), NaCl (0.8 g/L), KCl (0.8 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), KH_2PO_4 (2.0 g/L), $Na_2HPO_4 \cdot 12H_2O$ (2.0 g/L), $MgSO_4$ (0.2 g/L), $FeSO_4 \cdot 7H_2O$ (0.001 g/L); 2 mL trace element stock solution composed of (g/L): $FeCl_3 \cdot 6H_2O$ (0.08 g/L), $ZnSO_4 \cdot H_2O$ (0.75 g/L), $COCl_2 \cdot 6H_2O$ (0.08 g/L), $CuSO_4 \cdot 5H_2O$ (0.075 g/L), $MgSO_4 \cdot H_2O$ (0.75 g/L), H_3BO_3 (0.15 g/L), $Na_2MoO_4 \cdot 2H_2O$ (0.05 g/L). Autoclave (121 °C, 20 min), and adjust the pH to 7.3 (pH of the Lake Hulupi water at the time of the study) with NaOH (10 M) or HCl (1 M) [2].

Lysogeny Broth (LB): for 2 L, composition: 20 g tryptone, 10 g yeast extract, and 20 g NaCl Autoclave (121 °C, 20 min), and adjust the pH to 7.3 (pH of the Lake Hulupi water at the time of the study) with NaOH (10 M) or HCl (1 M) [3].

Counting agar plates: 30 g commercial microbial agar (plate count agar HIMEDIA Ref M091-500 g, Mumbai, India) in 2 L distilled water. Autoclaved (121 °C, 20 min), cooled at 40 °C, and poured in the petri dishes.

7. Scanning Electronic Microscope (SEM) Images of the Isolated Bacteria



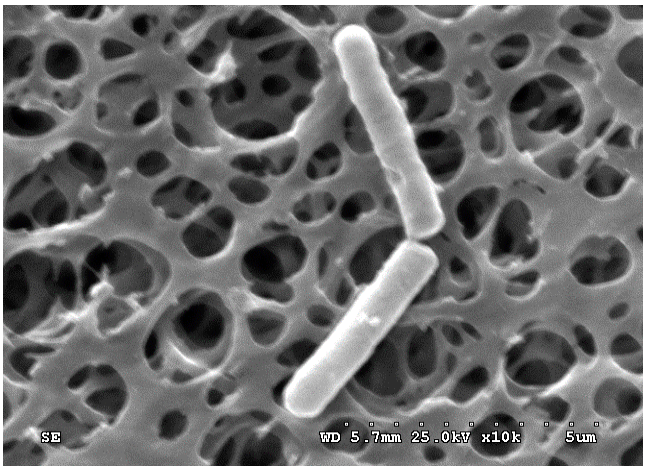


Figure S6. SEM images of the isolated bacteria *Bacillus* sp. After washing, the bacteria were completely dried using 99% ethanol and CO_2, respectively, before being examined under SEM in order to avoid the presence of any artefacts.

8. *Bacillus* sp. Incubation along with MC-LR

A decrease was observed in the *Bacillus* sp. population after 12 days (Figure S7) when the bacteria were incubated with no carbon source in MSM medium. However, the presence of MC-LR as a carbon source allowed a slight decrease in the bacteria population from 8.3×10^6 CFU/mL to 8×10^5 CFU/mL, and the difference in *Bacillus* sp. growth with or without MC-LR was not statistically significant for an exact $p = 0.049$. However, previous studies stipulated that bacteria may use MC-LR as their carbon source [4,5].

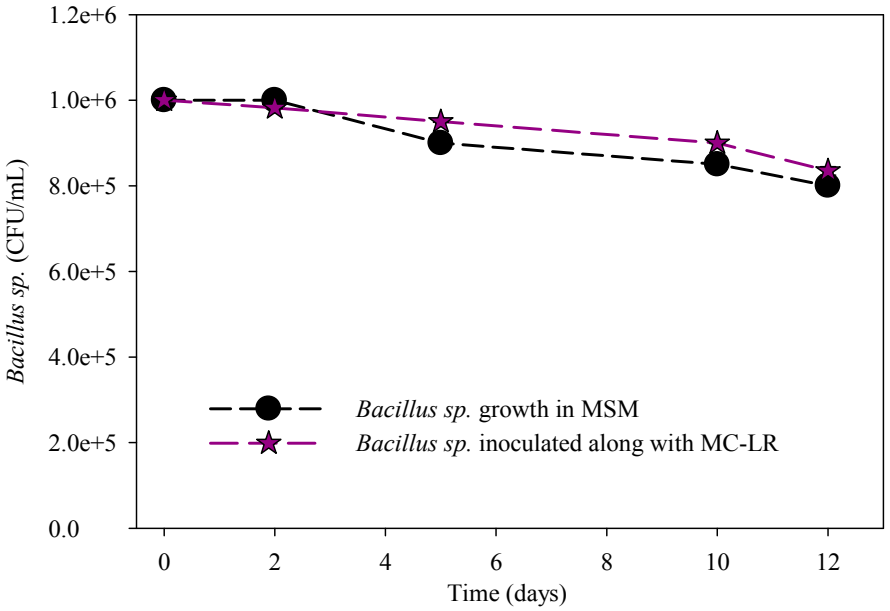


Figure S7. *Bacillus* sp. growth inoculated with and without MC-LR. The error bars represent one standard deviation for three measurements.

References

1. Atlas, R.M. *Handbook of Microbiological Media*; CRC Press: Boca Raton, FL, USA, 2010.
2. Sepahi, A.A.; Golpasha, I.D.; Emami, M.; Nakhoda, A. Isolation and characterization of crude oil degrading *Bacillus* spp. *J. Environ. Health Sci. Eng.* **2008**, *5*, 149–154.
3. Manage, P.M.; Edwards, C.; Singh, B.K.; Lawton, L.A. Isolation and identification of novel microcystin-degrading bacteria. *Appl. Environ. Microbiol.* **2009**, *75*, 6924–6928.
4. Wang, G.; Wu, J.; Xie, W.; Li, Y.; Jia, R. screening and identification of a microcystin-degrading bacterium strain and its enzymatic degradation of microcystin-LR by intracellular extract of bacillus cereus. *Wei Sheng Wu Xue Bao* **2012**, *52*, 96–103.
5. Zhang, J.; Shi, H.; Liu, A.; Cao, Z.; Hao, J.; Gong, R. Identification of a new microcystin-degrading bacterium isolated from lake Chaohu, China. *Bull. Environ. Contam. Toxicol.* **2015**, *94*, 661–666.