Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.




## Supporting Information

Table S1 – Bacterial isolates capable of degrading multiple microcystin variants.

| **Organism** | **Reference (s)** | **Cyanotoxins degraded** | **Presence of *mlr*A, *mlr*B, *mlr*C, *mlr*D** |
|---|---|---|---|
| *Arthrobacter* sp. C6, F7, R4 | Manage et al. (2009); Lawton et al. (2011) | MC-LR, LY, LF, LW, RR, Nodularin | Not detected |
| *Bordetella sp.* | Yang et al. (Yang et al. 2014a) | MC-LR, RR | *mlrA* |
| *Brevibacterium* sp. F3 | Manage et al. (2009); Lawton et al. (2011) | MC-LR, LY, LF, LW, RR, Nodularin | Not detected |
| *Sphingomonas sp.* MD1 | Saitou et al. (2003) | MC-LR, RR, YR | Unknown |
| *Methylobacillus* sp. | Hu et al. (2009) | MC-LR,RR | Unknown |
| *Paucibacter toxinivorans* 2C20 | Rapala et al. (2005) | MC-LR, MC-YR, Nodularin | Unknown |
| *Poterioochromonas* sp. | Ou et al. (2005); Zhang et al. (2008) | MC-LR,RR | Unknown |
| *Ralstonia solanacearum* | Yan et al. (2004) | MC-LR,RR | Unknown |
| *Rhodococcus* sp. C1 | Manage et al. (2009); Lawton et al. (2011) | MC-LR, LY, LF, LW, RR Nodularin | Not detected |
| *Sphingomonas* sp. 7CY | Ishii et al. (2004) | MC-LR, LY, LF, LW, RR Nodularin-Har | Unknown |
| *Sphingomonas* sp. B9 | Harada et al. (2004); Imanishi et al. (2005); Tsuji et al. (2006); Kato et al. (2007) | MC-LR, RR, Nodularin | Unknown |
| *Sphingosinicella microcystinovorans* sp. MDB2, MDB3 | Maruyama et al. (2006) | Unknown | Unknown |
| *Sphingosinicella microcystinovorans* sp. Y2 | Park et al. (2001); | MC-LR, YR, RR | Unknown |
| *Sphingomonas* sp. ACM-3962 | Bourne et al. (1996, 2001) | MC-LR, RR | *mlrA*, *mlrB*, *mlrC* and *mlrD* |
| *Sphingopyxis* sp. LH21 | Ho et al. (2007) | MC-LR, LA | *mlrA*, *mlrB*, *mlrC* and *mlrD* |
| *Sphingopyxis* sp. USTB-05 | Wang et al. (2010); Zhang et al. (2010); Yan et al. (2012); Wang et al. (2014) | MC-RR, LR | *mlrA, mlrB*, *mlrC* and *mlrD* |
| *Sphingopyxis* sp. TT25 | Ho et al. (2012) | MC-LR, RR, YR, LA, CYN, geosmin | *mlrA* |
| *Sphingopyxis* sp. C-1 | Okano et al. (2010) | MC-LR,RR | *mlrA* |
| *Stenotrophomonas acidaminiphila* sp. MC-LTH2 | Yang et al.(Yang et al. 2014b) | MC-LR,RR | Not detected |

**Text S1. Procedure for sequencing the 16S ribosomal RNA (rRNA) PCR fragment**

Sequencing reactions were performed in 50 µl volumes containing 10 ng of DNA, 25 µM each primer, 200 µM of each deoxynucleoside triphosphates (dNTPs), 1 mM MgCl2 and 2.5 units of rTaq DNA polymerase (GE Healthcare, Baie d'Urfé, QC) in 10X Taq polymerase buffer (100 mM Tris-Cl pH 9.0, 500 mM KCl, 15 mM $MgCl_2$). PCR amplification conditions involved an initial denaturation at 96°C for 5 min, followed by 30 cycles of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C. The 16S rRNA PCR products were purified with GFX™ PCR DNA and Gel Band Purification kit (GE Healthcare), quantified with a dilution of the 1 kb marker from MBI Fermentas (Amherst, NY, U.S.A.), SYBR Safe staining (Molecular Probes, Eugene, Oregon, U.S.A.) and spot densitometry using a ChemiImager (Alpha Innotech Corporation, San Leandro, CA). Sequencing of both strands was performed by the Plate-forme d'analyses biomoléculaires of University Laval, with the F1, F1b (complementary of R2), F2, R2, R13 primers (Dorsch and Stackebrandt 1992) and R14b primer from Laramée et al. (Laramée et al. 2000). The 16S rRNA gene sequence of each isolate was compared to the NCBI database using BLASTN (Altschul et al. 1990).

**Text S2. Genomic analysis**

Genes encoding for *cpn60* chaperonins (Hemmingsen et al. 1988) and *rpoA* alpha subunit of RNA polymerase (Galisa et al. 2012, Lemeille et al. 2005) and proteins involved in the degradation of microcystin, *mlrA*, *mlrB*, *mlrC*, and *mlrD* were identified with searches using the blastx program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the *cpn*DB sequence database (http://www.cpndb.ca/cpnDB/home.php) and custom databases composed of selected *mlr* sequences from nr (http://blast.ncbi.nlm.nih.gov/Blast.cgi). E value cutoffs of 1e-8 were used in all cases. To fully annotate the 17.9 kb sequence contig that contained all four *mlr* genes, open

reading frames (ORFs) were identified with Prodigal (Hyatt et al. 2010) using the *meta* procedure.

**Test S3. Transcriptomic analyses**

Assembled and unassembled sequences were then quality controlled and freed of remaining adapter sequences using Trimmomatic (Bolger et al. 2014), with the following settings: ILLUMINACLIP:2:30:10 LEADING:30 TRAILING:30 SLIDINGWINDOW:4:30 MINLEN:50 Transcripts harboring the *cpn60* reference gene and *mlr* genes were identified using blastn searches (1e-20) against the strain MB-E gene sequences identified as described above and counts were compiled for each dataset. Transcripts harboring the *rpoA* reference gene were also compared against strain MB-E gene sequences. Double counts resulting from the presence of the same gene on unassembled sequence pairs were identified using custom scripts, and dataset-wide counts were adjusted accordingly. Ratios of *mlr*/*cpn60* and *mlr*/*rpoA* gene expression were then calculated in order to normalize each dataset to a value approximating the average single cell expression profile. The latter numbers were averaged between replicate datasets for each time point of both culturing conditions (MCLR and related toxin mix).

**Text S4. Analytical method for cyanotoxin measurement**

Residual concentrations of cyanotoxins in the biodegradation experiments were monitored by a system consisting of an HTC thermopal autosampler (CTC analytics AG, Zwingen, Switzerland) with a 1 ml loop, a dual switching-column array and a liquid chromatography tandem mass spectroctometry system. A quaternary pump Accela 600 (Thermo Finnigan, San Jose, CA) was used for sample loading onto an on-line Hypersil Gold C18 column (20 mm x 2.1 mm, 12 μm particle size). The column switching system was made of a six-port and a ten-port

valve (VICI® Valco Instruments Co. Inc., Houston, TX). The switching process has been described previously (Viglino et al. 2008).

The elution was performed using a quaternary pump Accela 1200 (Thermo Finnigan, San Jose, CA) and the chromatographic separation was done with a Hypersil Gold column (100 mm X 2.1 mm, 1.9 µm particle size) kept at 55°C in a thermostated column compartment. The total run time was 8 minutes. The analytical column was preceded by a guard column (2 X 2mm, 5mm) of the same packing material. Gradient conditions for the on-line SPE loading pump and analytical pump are provided in Table S2.

A TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA) with a Heated Electrospray (HESI) source was used for detection and quantification. The mass spectrometer was operated in selected reaction monitoring (SRM) mode and the ionization was in the positive mode. The initial compound-dependent parameters for MS and MS/MS optimization conditions are presented in Table S3. The source-dependent parameters were as follows: spray voltage (3200 V), vaporizer temperature (450 °C), sheath gas pressure (35 arbitrary units), auxiliary gas pressure (10 arbitrary units) and capillary temperature (350 °C). The scan time was adjusted to 0.02 s. The first and third quadrupole were operated at 0.7 Da FWHM and the collision gas pressure of the second quadrupole was set at 1.5 mTorr. The limits of detection (LOD) were in range of 10-50 ng $L^{-1}$ (Table S3).

**Table S2**.Valve program, on-line SPE (loading pump) and LC (analytical pump) gradient elution conditions used for the pre-concentration and separation of selected cyanotoxins.

| Loading pump (to six port valve) | | | | | Analytical pump (to ten port valve) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Time (min) | A (%) | B (%) | Flow rate (µL min$^{-1}$) | Time (min) | A (%) | B (%) | C (%) | Flow rate (µL min$^{-1}$) | |
| On-line SPE loading step | 0 | 100 | 0 | 500 | 0 | 75 | 25 | 0 | 525 | Column re-equilibration |
| | 2.25 | 100 | 0 | 500 | 2.25 | 75 | 25 | 0 | 525 | |
| | | | | | 2.50 | 45 | 55 | 0 | 525 | |
| | 2.27 | 0 | 100 | 1500 | 3.30 | 45 | 55 | 0 | 525 | Elution and chromatographic separation |
| Loop wash | | | | | 3.31 | 55 | 0 | 45 | 525 | |
| | 7.30 | 0 | 100 | 1500 | 5.10 | 55 | 0 | 45 | 525 | |
| | | | | | 6.80 | 5 | 0 | 95 | 525 | |
| SPE column conditioning | 7.31 | 100 | 0 | 1500 | 6.81 | 75 | 25 | 0 | 525 | Column re-equilibration |
| | 8.00 | 100 | 0 | 1500 | 8.00 | 75 | 25 | 0 | 525 | |

A : $H_2O$ +0.1% formic acid

B : Methanol + 0.1% formic acid

C : Acetonitrile + 0.1% formic acid

**Table S3.** MS/MS optimized parameters for all selected compounds.

| **Compound** | **Precursor ion (m/z)** | **Product ion (m/z)** | **TL (V)** | **CE (eV)** | **LOD (ng/l)** |
|---|---|---|---|---|---|
| **Anatoxin** | 166 | 149 | 86 | 11 | 50 |
| | $[M+H]^+$ | 120 | 86 | 13 | |
| **Cylindrospermopsin** | 416 | 194 | 151 | 37 | 50 |
| | $[M+H]^+$ | 176 | 151 | 31 | |
| **MC-RR** | 520 | 135 | 138 | 31 | 10 |
| | $[M+2H]^{2+}$ | 105 | 138 | 47 | |
| **MC-YR** | 1046 | 135 | 183 | 58 | 10 |
| | $[M+H]^+$ | 213 | 183 | 58 | |
| **MC-LR** | 996 | 135 | 188 | 57 | 10 |
| | $[M+H]^+$ | 213 | 188 | 39 | |
| **MC-LY** | 1003 | 265 | 118 | 50 | 20 |

| | | | | | |
|---|---|---|---|---|---|
| | $[M+H]^+$ | 135 | 118 | 37 | |
| **MC-LW** | 1026 | 891 | 164 | 24 | 20 |
| | $[M+H]^+$ | 583 | 164 | 29 | |
| **MC-LF** | 987 | 213 | 150 | 34 | 20 |
| | $[M+H]^+$ | 375 | 150 | 22 | |
| **Nodularin*** | 825 | 135 | 148 | 50 | - |

*Internal standard

**Table S4.** Pseudo-first-order rate constants (K) and linear regression coefficients of cyanobacteria toxins biodegradation by *Sphingopyxis* sp. MB-E.

| **Toxin** | **Rate Constant ($h^{-1}$)** | **$R^2$** |
|---|---|---|
| MCLR | 0.33 | 0.887 |
| MCYR | 0.39 | 0.894 |
| MCLY | 0.33 | 0.694 |
| MCLW | 0.39 | 0.799 |
| MCLF | 0.29 | 0.813 |

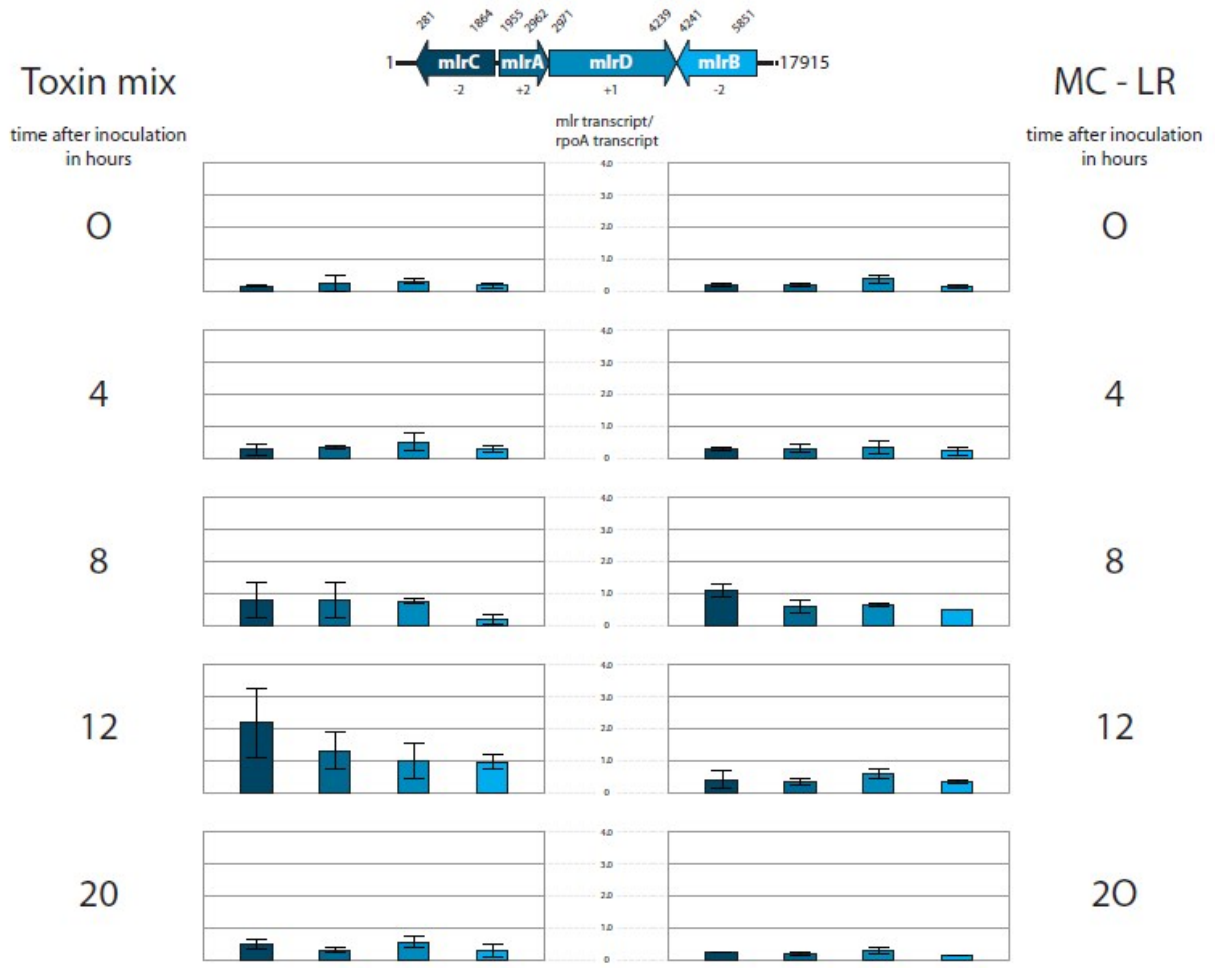


Figure S1. Expression of the *mlr* gene cluster during growth of *Sphingopyxis* sp. MB-E on microcystin LR and the mixture of five cyanotoxins, in comparison to the expression of the *rpoA* reference gene. Error bars represent the standard deviation of duplicate samples and duplicate analyses.

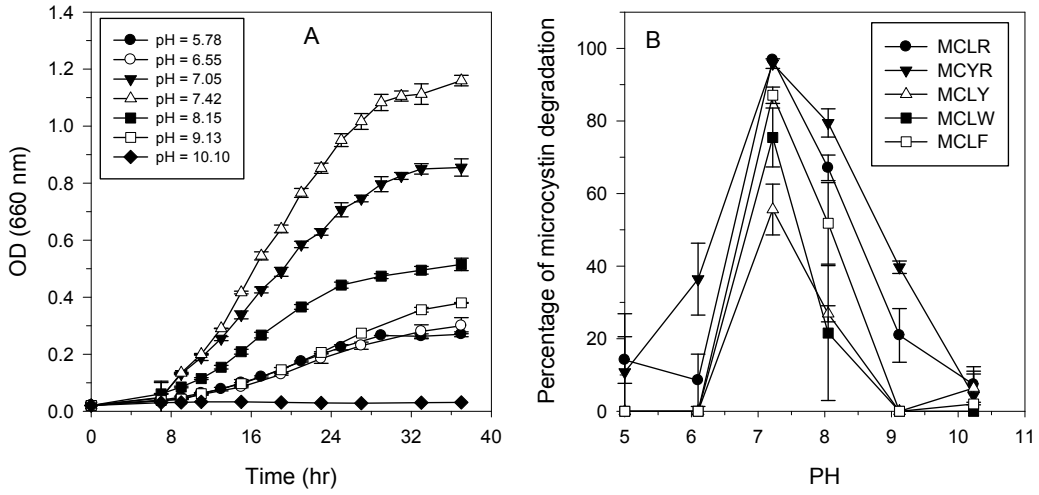


**Figure S2.** Effect of initial pH on the growth of *Sphingopyxis* sp MB-E (A); and on microcystin degradation activity (B). Degradation activity was determined as percentage of initial microcystin concentration removal after 7 h. The error bars indicate the standard deviation of triplicate samples and duplicate analyses.

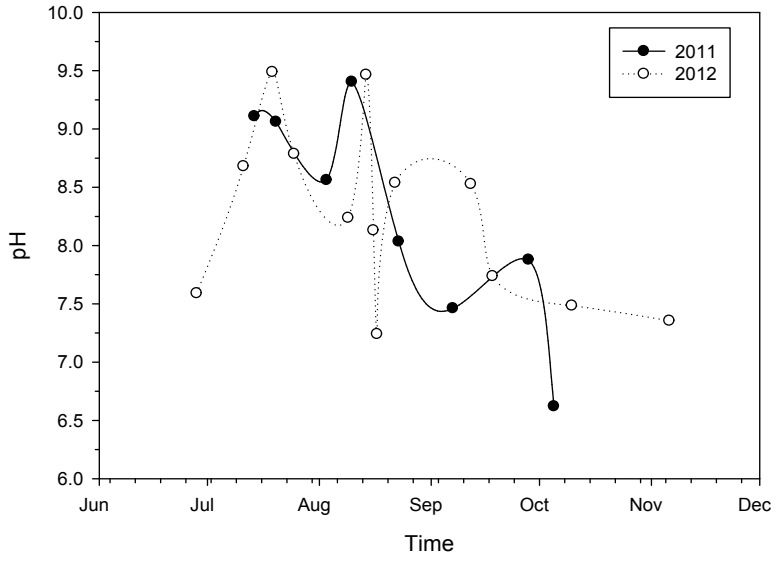


**Figure S3.** Average pH in the littoral area of Philipsburg in Missisquoi Bay water in 2011 and 2012.

**References**

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic local alignment search tool. Journal of Molecular Biology 215(3), 403-410.
Bolger, A.M., Lohse, M. and Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15), 2114-2120.
Bourne, D.G., Jones, G.J., Blakeley, R.L., Jones, A., Negri, A.P. and Riddles, P. (1996) Enzymatic pathway for the bacterial degradation of the cyanobacterial cyclic peptide toxin microcystin LR. Applied and Environmental Microbiology 62(11), 4086-4094.
Bourne, D.G., Riddles, P., Jones, G.J., Smith, W. and Blakeley, R.L. (2001) Characterisation of a gene cluster involved in bacterial degradation of the cyanobacterial toxin microcystin LR. Environmental Toxicology 16(6), 523-534.
Dorsch, M. and Stackebrandt, E. (1992) Some modifications in the procedure of direct sequencing of PCR amplified 16S rDNA. Journal of Microbiological Methods 16(4), 271-279.
Galisa, P.S., da Silva, H.A.P., Macedo, A.V.M., Reis, V.M., Vidal, M.S., Baldani, J.I. and Simões-Araújo, J.L. (2012) Identification and validation of reference genes to study the gene expression in *Gluconacetobacter diazotrophicus* grown in different carbon sources using RT-qPCR. Journal of Microbiological Methods 91(1), 1-7.
Harada, K.-i., Imanishi, S., Kato, H., Mizuno, M., Ito, E. and Tsuji, K. (2004) Isolation of Adda from microcystin-LR by microbial degradation. Toxicon 44(1), 107-109.
Hemmingsen, S.M., Woolford, C., van der Vies, S.M., Tilly, K., Dennis, D.T., Georgopoulos, C.P., Hendrix, R.W. and Ellis, R.J. (1988) Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature 333(6171), 330-334.
Ho, L., Hoefel, D., Saint, C.P. and Newcombe, G. (2007) Isolation and identification of a novel microcystin-degrading bacterium from a biological sand filter. Water Research 41(20), 4685-4695.
Ho, L., Tang, T., Monis, P.T. and Hoefel, D. (2012) Biodegradation of multiple cyanobacterial metabolites in drinking water supplies. Chemosphere 87(10), 1149-1154.
Hu, L.B., Yang, J.D., Zhou, W., Yin, Y.F., Chen, J. and Shi, Z.Q. (2009) Isolation of a *Methylobacillus* sp. that degrades microcystin toxins associated with cyanobacteria. New Biotechnology 26(3â€“4), 205-211.
Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W. and Hauser, L.J. (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC bioinformatics 11(1), 119.
Imanishi, S., Kato, H., Mizuno, M., Tsuji, K. and Harada, K.-i. (2005) Bacterial degradation of microcystins and nodularin. Chemical Research in Toxicology 18(3), 591-598.
Ishii, H., Nishijima, M. and Abe, T. (2004) Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38(11), 2667-2676.
Kato, H., Imanishi, S.Y., Tsuji, K. and Harada, K.-i. (2007) Microbial degradation of cyanobacterial cyclic peptides. Water Research 41(8), 1754-1762.
Laramée, L., Lawrence, J.R. and Greer, C.W. (2000) Molecular analysis and development of 16S rRNA oligonucleotide probes to characterize a diclofop-methyl-degrading biofilm consortium. Canadian Journal of Microbiology 46(2), 133-142.
Lawton, L., Welgamage, A., Manage, P. and Edwards, C. (2011) Novel bacterial strains for the removal of microcystins from drinking water. Water Science & Technology 63(6), 1137-1142.

Lemeille, S., Latifi, A. and Geiselmann, J. (2005) Inferring the connectivity of a regulatory network from mRNA quantification in *Synechocystis* PCC6803. Nucleic Acids Research 33(10), 3381-3389.
Manage, P.M., Edwards, C., Singh, B.K. and Lawton, L.A. (2009) Isolation and identification of novel microcystin-degrading bacteria. Applied and environmental microbiology 75(21), 6924-6928.

Maruyama, T., Park, H.-D., Ozawa, K., Tanaka, Y., Sumino, T., Hamana, K., Hiraishi, A. and Kato, K. (2006) *Sphingosinicella microcystinivorans* gen. nov., sp. nov., a microcystin-degrading bacterium. International Journal of Systematic and Evolutionary Microbiology 56(1), 85-89.
Okano, K., Shimizu, K., Kawauchi, Y., Maseda, H., Utsumi, M., Zhang, Z., Neilan, B.A. and Sugiura, N. (2010) Characteristics of a microcystin-degrading bacterium under alkaline environmental conditions. Journal of toxicology 2009, 8.
Ou, D., Song, L., Gan, N. and Chen, W. (2005) Effects of microcystins on and toxin degradation by *Poterioochromonas* sp. Environmental Toxicology 20(3), 373-380.
Park, H.D., Sasaki, Y., Maruyama, T., Yanagisawa, E., Hiraishi, A. and Kato, K. (2001) Degradation of the cyanobacterial hepatotoxin microcystin by a new bacterium isolated from a hypertrophic lake. Environmental Toxicology 16(4), 337-343.
Rapala, J., Berg, K.A., Lyra, C., Niemi, R.M., Manz, W., Suomalainen, S., Paulin, L. and Lahti, K. (2005) *Paucibacter toxinivorans* gen. nov., sp. nov., a bacterium that degrades cyclic cyanobacterial hepatotoxins microcystins and nodularin. International Journal of Systematic and Evolutionary Microbiology 55(4), 1563-1568.
Saitou, T., Sugiura, N., Itayama, T., Inamori, Y. and Matsumura, M. (2003) Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. Aqua 52, 13-18.
Tsuji, K., Asakawa, M., Anzai, Y., Sumino, T. and Harada, K.-i. (2006) Degradation of microcystins using immobilized microorganism isolated in an eutrophic lake. Chemosphere 65(1), 117-124.
Viglino, L., Aboulfadl, K., Prévost, M. and Sauvé, S. (2008) Analysis of natural and synthetic estrogenic endocrine disruptors in environmental waters using online preconcentration coupled with LC-APPI-MS/MS. Talanta 76(5), 1088-1096.
Wang, H., Yan, H., Ma, S., Liu, X., Yin, C., Wang, H., Xu, Q. and Lv, L. (2014) Characterization of the second and third steps in the enzymatic pathway for microcystin-RR biodegradation by *Sphingopyxis* sp. USTB-05. Annals of Microbiology 65(1), 495-502.
Wang, J., Wu, P., Chen, J. and Yan, H. (2010) Biodegradation of microcystin-RR by a new isolated *Sphingopyxis* sp. USTB-05. Chinese Journal of Chemical Engineering 18(1), 108-112.
Yan, H., Pan, G., Zou, H., Li, X. and Chen, H. (2004) Effective removal of microcystins using carbon nanotubes embedded with bacteria. Chinese Science Bulletin 49(16), 1694-1698.
Yan, H., Wang, J., Chen, J., Wei, W., Wang, H. and Wang, H. (2012) Characterization of the first step involved in enzymatic pathway for microcystin-RR biodegraded by *Sphingopyxis* sp. USTB-05. Chemosphere 87(1), 12-18.
Yang, F., Zhou, Y., Sun, R., Wei, H., Li, Y., Yin, L. and Pu, Y. (2014a) Biodegradation of microcystin-LR and-RR by a novel microcystin-degrading bacterium isolated from Lake Taihu. Biodegradation 25(3), 447-457.
Yang, F., Zhou, Y., Yin, L., Zhu, G., Liang, G. and Pu, Y. (2014b) Microcystin-degrading activity of an indigenous bacterial strain *Stenotrophomonas acidaminiphila* MC-LTH2 Isolated from Lake Taihu. PLoS ONE 9(1), e86216.

Zhang, M., Pan, G. and Yan, H. (2010) Microbial biodegradation of microcystin-RR by bacterium *Sphingopyxis* sp. USTB-05. Journal of Environmental Sciences 22(2), 168-175.
Zhang, X., Hu, H.-Y., Hong, Y. and Yang, J. (2008) Isolation of a Poterioochromonas capable of feeding on *Microcystis aeruginosa* and degrading microcystin-LR. FEMS Microbiology Letters 288(2), 241-246.