

# MICROCYSTIN CHANGES AND PROTEOMIC RESPONSES OF *MICROCYSTIS AERUGINOSA* EXPOSED TO CADMIUM DURING COLONY FORMATION

BI, X. D.<sup>1\*</sup> – DAI, W.<sup>1\*</sup> – SUN, J. S.<sup>2</sup> – JIA, N.<sup>3</sup>

<sup>1</sup>Key Laboratory of Aquatic-Ecology and Aquaculture of Tianjin, Department of Fisheries Sciences, Tianjin Agricultural University  
No. 22 Jinjing Road, Xiqing District, Tianjin 300384, China

<sup>2</sup>Tianjin Key Laboratory of Animal and Plant Resistance, Tianjin Normal University  
No. 393 Binshui West Road, Xiqing District, Tianjin 300387, China  
(phone: +86-022-2376-6539; fax: +86-022-2376-6000)

<sup>3</sup>Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University  
No. 92 Weijin Road, Nankai District, Tianjin 300372, China  
(phone: +86-022-2740-3902; fax: +86-022-2740-3902)

\*Corresponding authors

e-mail: yl80123@aliyun.com (X. D. Bi), daiweitj@126.com (W. Dai); phone: +86-022-2378-1299; fax: +86-022-2378-8970

(Received 1<sup>st</sup> Mar 2019; accepted 21<sup>st</sup> May 2019)

**Abstract.** *Microcystis* occurs as colonies in natural waters and always disaggregates into unicellular cells in the laboratory. Cadmium (Cd) could stimulate *Microcystis* colony formation in the laboratory. To elucidate the mechanism involved in cadmium-induced colony formation of *Microcystis*, microcystin (MC) changes and proteomic responses in *M. aeruginosa* treated with seven concentrations of Cd (0, 0.0125, 0.0250, 0.0500, 0.1000, 0.2000 and 0.4000 mg/L) for 40 h were studied. The results showed that seven concentrations of Cd except 0.4000 mg/L Cd could significantly induce colony formation ( $P < 0.05$ ), and the Cd-induced colony-stimulating effects were accompanied with the increased intracellular MC-RR production. A total of 1170 proteins, including seven MC biosynthesis-related enzymes and seven polysaccharide biosynthesis-related enzymes, were identified using 2D-LC-MS/MS combined with isobaric tags for relative and absolute quantitation (iTRAQ). 0.4000 mg/L Cd could downregulate all the identified enzymes involved in MC and polysaccharide biosynthesis while most of these enzymes were upregulated during Cd-induced colony formation. Cd might stimulate *Microcystis* colony formation via increasing the biosynthesis of MC and polysaccharide.

**Keywords:** cyanotoxin, polysaccharide, heavy metal ions, iTRAQ, Cyanobacteria colony

## Introduction

Cyanobacteria blooms have been paid more and more attention all over the world for their toxic impacts on freshwater environments and human health (O'Neil et al., 2012). *Microcystis* is a common cyanobacterial genus forming blooms (Shen and Song, 2007). In natural freshwaters, *Microcystis* cells always aggregate into colonies (Zhu et al., 2016). Colony formation provides *Microcystis* with a competitive advantage in preventing zooplankton grazing, viral and bacterial attack (Yang and Kong, 2012). However, the mechanism involved in *Microcystis* colony formation remains poorly understood.

In laboratory cultures, *Microcystis* is hard to keep colonial phenotype and colonial *Microcystis* usually disaggregates into single cells or a few paired cells (Huisman et al.,

2018). Previous studies have identified that zooplankton grazing pressure (Yang et al., 2012), ultraviolet radiation (Sommaruga et al., 2008), heterotrophic bacteria cocultivation (Shen et al., 2011), low light intensities and temperatures (Li et al., 2013) could stimulate colony formation of *Microcystis* cultured in the laboratory. Moreover, we found that the contents of lead (Pb), cadmium (Cd), chromium (Cr), aluminium (Al), ferrum (Fe), and manganese (Mn) in *Microcystis* cell were significantly positive with the colony size in natural freshwaters (Bi et al., 2015). Cd(II) could promote *Microcystis* colony formation in an appropriate concentration range (Bi et al., 2016). Moreover, Gan et al. (2012) found that microcystin (MC, a typical cyanotoxin), supplementation in medium could enhance the size of *Microcystis* colony and depletion of MC could dramatically decrease colony size. Different from above external biotic and abiotic environmental factors, MC released by toxic *Microcystis* might act as an internal signal factor influencing the aggregation of *Microcystis* cells.

Proteomics, the large-scale analysis of proteins, can link the upstream genome and the downstream metabolome (Wilmes and Bond, 2009). Proteomic analysis has been widely applied in reflecting functional protein dynamic responses of cyanobacteria under stress (Alexova et al., 2016). According to the proteomic responses of *M. aeruginosa* to N or P starvation, Yue et al. (2015) found that N limitation enhanced the expression of several proteins relating to cellular C metabolism and fixation while P limitation downregulated the proteins involved in protein synthesis and the assimilation of C and N. Proteomic response of *M. aeruginosa* revealed that long-term exposure of amoxicillin could affect the cellular biosynthesis process and the metabolism of nucleoside phosphate and carbohydrate (Liu et al., 2016).

To make clear how heavy metals affect colony formation of *Microcystis*, *M. aeruginosa* were treated with seven concentrations of Cd for 40 h, and their changes in the colony formation, intracellular MC contents and proteomic responses were investigated. The results will help us reveal the impact of heavy metals on the breakout of *Microcystis* blooms in natural waters.

## Materials and methods

### *Algal culture conditions*

*M. aeruginosa* FACHB-905 was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences. Algae were cultured in BG11 medium (without EDTA) under a L:D (12 h: 12 h) cycle at a light intensity of 40  $\mu\text{mol}/\text{m}^2/\text{s}$  at 25 °C. Stock solution of  $\text{CdCl}_2$  (Merck, Germany) was added to algae cultured to the exponential phase, and the final concentrations of Cd were prepared as follows: 0.0125 (Cd\_A), 0.0250 (Cd\_B), 0.0500 (Cd\_C), 0.1000 (Cd\_D), 0.2000 (Cd\_E) and 0.4000 mg/L (Cd\_F). The culture without Cd was used as control group (CK). All groups were done in triplicate, and the initial density of *M. aeruginosa* was  $5.2 \times 10^6$  ind/L. After 40 h, samples were taken from algal cultures to study changes in cell densities, colony numbers, MC content and proteomic analysis.

### *Counting algal cell density and colony*

Algal cell density and colony were counted using a hemocytometer under microscope. Samples were collected 1 cm below the medium surface, and the flasks

were shaken up slightly before sampling. Algal phenotype was classified as unicellular, two-cell aggregation and colony (aggregation of  $\geq 3$  cells).

### ***Intracellular MC extraction and analysis***

Intracellular MC was extracted and measured according to the method of Bi et al. (2017) using high-performance liquid chromatography (SPD-M20A, Shimadzu, Japan) equipped with a Shim-Pack VP-ODS column (250 mm  $\times$  4.6 mm). MC-RR was identified by its characteristic UV spectra and retention time, and spiked with purified standards of MC-RR (Sigma, USA).

### ***Proteomic analysis***

#### ***Sampling, proteins extraction and iTRAQ labeling***

Samples were extracted with Lysis buffer (8 M Urea, 40 mM Tris-HCl or TEAB, pH 8.5) containing 2 mM EDTA and 1mM PMSF, and then added with 10 mM DTT after being kept on ice for 5 min, then were sonicated at 200 W for 1 min. After centrifugation (25000 g, 4 °C) for 20 min, the supernatants obtained were incubated at 56 °C for 1 h and cooled to room temperature, then incubated with 55 mM IAM (for alkylation) in darkness for 45 min, and then centrifuged (25000 g, 4 °C) for 20 min. Protein concentrations were quantified using Bradford method. The protein solution (100  $\mu$ g) with 8 M urea was diluted 4 times with 100 mM TEAB. For iTRAQ analysis, proteins were digested with Trypsin Gold (Promega, Madison, WI, USA) with a protein-to-trypsin ratio of 40:1 at 37 °C overnight, and peptides were desalted with a Strata X C18 column and vacuum-dried. The peptides were dissolved in 30  $\mu$ l 0.5 M TEAB via vortexing. ITRAQ labeling reagents recovered to ambient were transferred and combined with proper samples. Peptide labeling was performed using ITRAQ Reagent 8-plex Kit. The labeled peptides with different reagents were combined and desalted with a Strata X C18 column and vacuum-dried, the above experimental method refers to Wiese et al. (2007).

#### ***Peptide fractionation, HPLC, mass spectrometer detection***

After being reconstituted with buffer A (5% ACN, 95% H<sub>2</sub>O, adjusting pH to 9.8 with ammonia) to 2 mL, labeled peptides were fractionated using a Shimadzu LC-20AB HPLC pump system equipped with a high pH RP column at a flow rate of 1 mL/min with a gradient of buffer B (5% H<sub>2</sub>O, 95% ACN, adjusting pH to 9.8 with ammonia): 5% for 10 min, 5-35% for 40 min, 35-95% for 1 min, 95% for 3 min, 95-5% within 1 min followed by 5% buffer for 10 min. Chromatograms were recorded at 214 nm, and fractions were collected at 1-min intervals. In total, 20 fractions were collected and vacuum-dried, dissolved in buffer (2% ACN, 0.1% FA in water) and centrifuged at 20,000 g for 10 min. Supernatants were loaded onto a C18 trap column using a LC-20AD nano-HPLC instrument (Shimadzu, Kyoto, Japan) and eluted, then separated with an analytical C18 column packed in-house at a flow rate of 300 nL/min with the following gradient of buffer (2% H<sub>2</sub>O and 0.1% FA in ACN): 8-35% in 35 min; 35-60% in 5 min, 80% for 5 min, 80-5% in 0.1 min and equilibrating for 10 min. Data was acquired with a Triple TOF 5600 System equipped with a Nanospray III source (SCIEX, Framingham, MA, USA) and a pulled quartz tip emitter (New Objectives, Woburn, MA). This procedure was carried out by Beijing Genomics Institute

(Shenzhen, China), the above experimental method refers to Olson et al. (2013) and Lyu et al. (2016).

#### *Protein identification, quantification and data analysis*

For an MS/MS Ions Search, each query represented a complete MS/MS spectrum. The common protein databases: NCBI Inr, SwissProt (Magrane and UniProt Consortium, 2011), and UniProt were used for protein identification. ITRAQ quantification was applied by BGI's IQuant (Wen et al., 2014). Proteins with 1.2-fold change and Q-value less than 0.05 were determined as differentially expressed protein. Genes were annotated by using BLAST\* (Altschul and Gish, 1996) against Clusters of Orthologous Groups of proteins database (COG) (Tatusov et al., 2003) and Kyoto Encyclopedia of Genes and Genomes database (KEGG) (Kanehisa et al., 2006). We set Cd\_A/CK, Cd\_B/CK, Cd\_C/CK, Cd\_D/CK, Cd\_E/CK, Cd\_F/CK, as comparison group.

#### *Statistical analysis*

Data expressed as means  $\pm$  SD were subjected to one-way analysis of variance (SPSS ver. 10.0) to determine differences among groups, and least significant difference multiple-range test was used to determine significance differences ( $P < 0.05$ ).

## **Results and discussion**

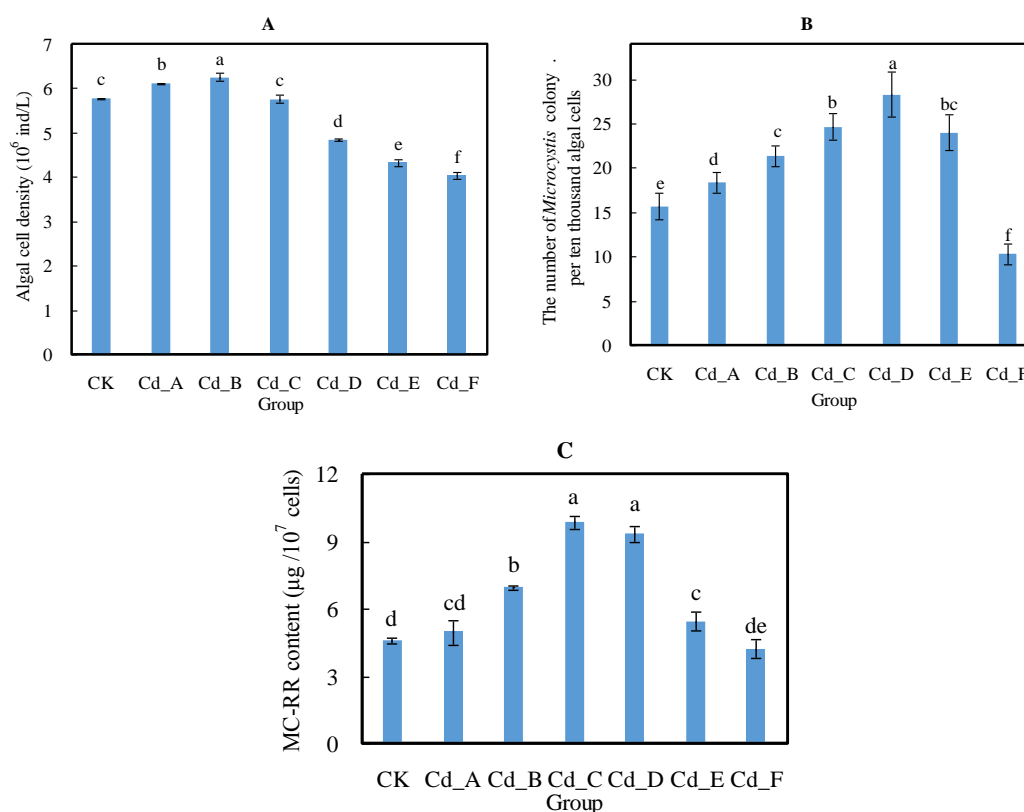
### ***Effects of Cd on the growth, colony formation and intracellular MC-RR content of *M. aeruginosa****

As shown in *Figure 1A*, with the increasing Cd concentrations, cell densities of *M. aeruginosa* increased first and then decreased, and the maximum cell density appeared in *M. aeruginosa* exposed to 0.0250 mg/L Cd. As compared to CK, all tested concentrations of Cd except 0.4000 mg/L Cd could significantly induce colony formation ( $P < 0.05$ ), and 0.1000 mg/L Cd showed the best inductive effects (*Fig. 1B*). Intracellular MC-RR contents of *M. aeruginosa* exposed to 0.0250, 0.0500, 0.1000 and 0.2000 mg/L of Cd increased significantly as compared to CK (*Fig. 1C*). Cd could contribute to *M. aeruginosa* cells aggregation, and the Cd-induced colony-stimulating effects accompanied with the increased intracellular MC-RR contents. In our previous research, it was observed that the *Microcystis* colonies with smallest size in natural waters had the highest MC cellular production which decreased with the increasing colony size (Bi et al., 2017), suggesting toxic *Microcystis* might play a vital role in *Microcystis* colony formation via MCs production. Based on our above results, we speculated that Cd could increase the production of MC which acted as an internal signal stimulating the colony formation.

### ***Proteomic analysis of *M. aeruginosa* exposed to Cd***

A total of 405114 spectrums were generated, 7766 peptides and 1170 protein were identified (*Table A1*). According to the distribution of COG classification, it was found that in *M. aeruginosa* FACHB-905 genome, the number of genes relating to energy production and conversion (C), amino acid transport and metabolism (E), carbohydrate transport and metabolism (G) and cell wall/membrane/envelope biogenesis (M) was more than that of other function related genes (*Table A2*). Among all the KEGG

pathways, biosynthesis of secondary metabolites, antibiotics and amino acids accounted for 22.46%, 14.08% and 9.42%, respectively (Table A3).

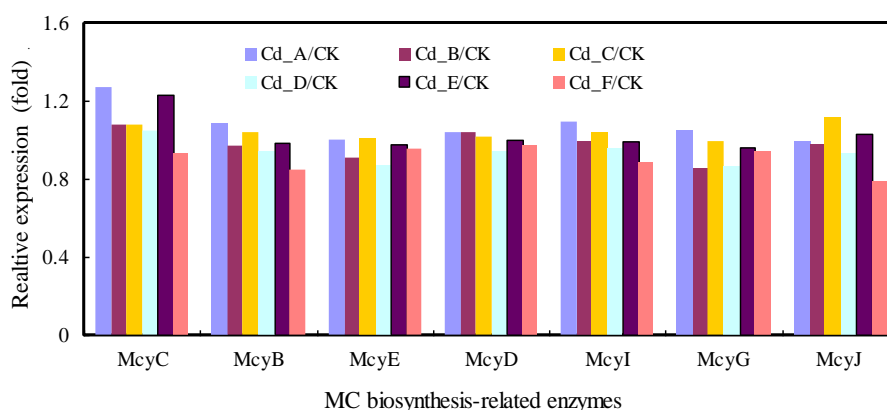


**Figure 1.** Effects of Cd on the growth (A), colony formation (B) and intracellular MC-RR content (C) of *M. aeruginosa*

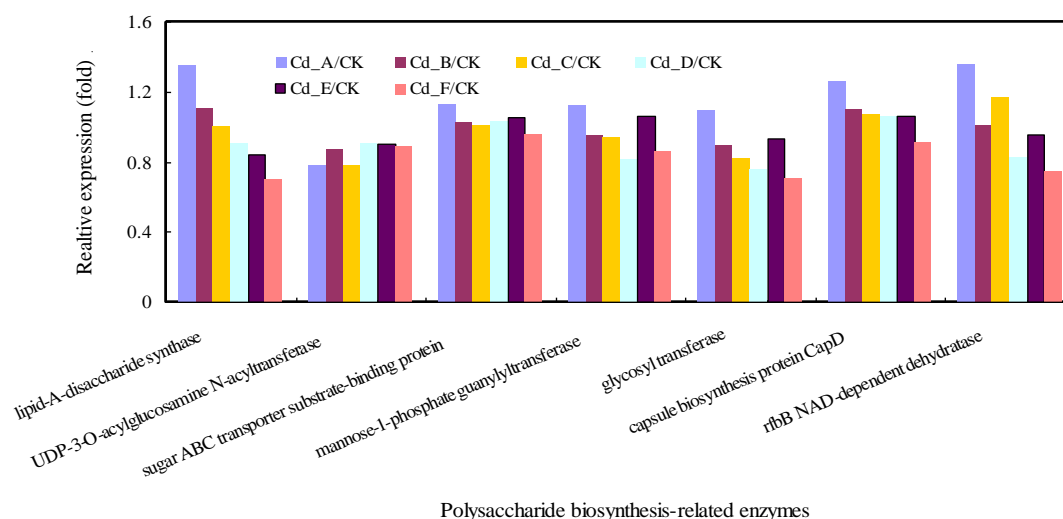
MCs are synthesized by a large, modular enzyme complex, including peptide synthetases McyA, McyB, and McyC, a polyketide synthase McyD, two hybrid enzymes (McyE and McyG) consisting of peptide synthetase and polyketide synthase modules, and enzymes putatively involved in the tailoring (McyJ, McyF, and McyI) and transport (McyH) of MCs (Tillett et al., 2000). As shown in Figure 2, a total of seven MC biosynthesis-related enzymes, including McyB, McyC, McyD, McyE, McyG, McyI, and McyJ were identified using proteomic analysis. Consistent with the changes in MC-RR, 0.4000 mg/L Cd downregulated all these seven identified MC biosynthesis-related enzymes. 0.050 mg/L Cd upregulated all identified MC biosynthesis-related enzymes except McyG, and 0.0125 mg/L Cd upregulated all identified MC biosynthesis-related enzymes except McyJ. Other concentrations of Cd regulated MC biosynthesis-related enzymes to different degree. All tested concentrations of Cd except 0.4000 mg/L Cd could upregulated the expression level of McyC while McyD expression of *M. aeruginosa* exposed to 0.0125-0.0500 mg/L Cd was upregulated (Table A4).

Both soluble and total carbohydrates in colonial *Microcystis* cells and sheaths were significantly higher than those in disaggregated cells (Zhang et al., 2007). In colonial *Microcystis*, extracellular polysaccharides (EPS) were the main constituents of the

sheaths relating to cell aggregation (Plude et al., 1991). Previous researches have proved that biotic and abiotic factors could stimulate polysaccharide production (Xu et al., 2016; Yang et al., 2007). We also found that the contents of intracellular polysaccharide and bound extracellular polysaccharide in Cd-induced *M. aeruginosa* colony increased significantly, which could stimulate aggregation of algal cell and eventually promote colony formation (Bi et al., 2016). Many genes were involved in polysaccharides synthesis pathway, such as *capD*, *csaB*, *tagH*, *rfbB*, and *epsL* (Thurlow et al., 2009). Proteomic analysis showed that all identified polysaccharide biosynthesis-related enzymes were downregulated in *M. aeruginosa* exposed to 0.4000 mg/L Cd which led to decreased colonies (Fig. 3). All concentrations of Cd except 0.4000 mg/L triggered upregulation of capsule biosynthesis protein CapD and sugar ABC transporter substrate-binding protein while *rfbB* NAD-dependent dehydratase and lipid-A-disaccharide synthase of *M. aeruginosa* exposed to 0.0125-0.0500 mg/L Cd were upregulated. These upregulated polysaccharide biosynthesis-related enzymes might be responsible for Cd-induced increase in polysaccharides.

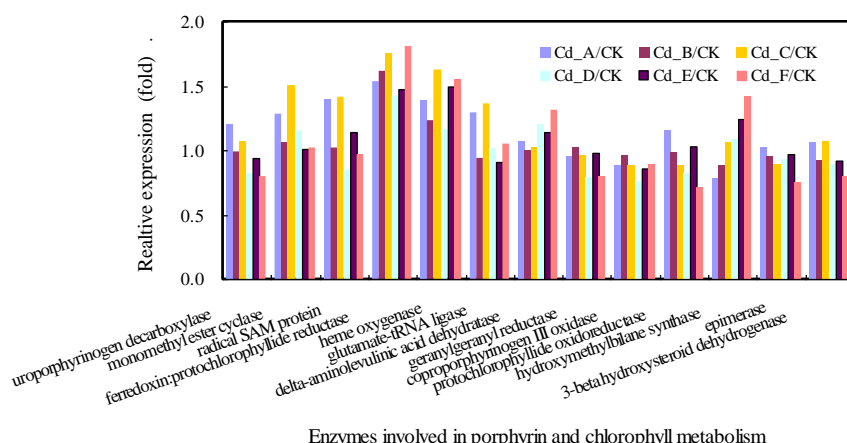


**Figure 2.** Cd-induced changes in the expressions of MC biosynthesis-related enzymes of *M. aeruginosa*



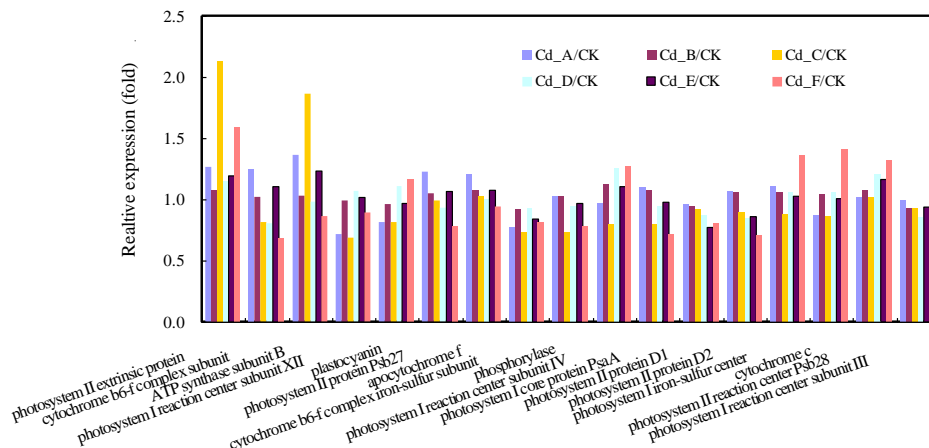
**Figure 3.** Cd-induced changes in the expressions of polysaccharide biosynthesis-related enzymes of *M. aeruginosa*

The strong competition advantages of *M. aeruginosa* in plankton system are based on its high photosynthetic activity (Amorim et al., 2017). Cd remarkably influenced the porphyrin and chlorophyll metabolism (Fig. 4), and photosynthesis (Fig. 5) in *M. aeruginosa*. 16 related proteins were upregulated or downregulated significantly in *M. aeruginosa* exposed to 0.4000 mg/L Cd. The expression level of heme oxygenase and ferredoxin: protochlorophyllide reductase increased in all groups, thus preventing or minimizing ROS-induced damages (Zhou et al., 2006). Furthermore, the expression level of magnesium-protoporphyrin IX monomethyl ester cyclase, radical SAM protein, glutamate-tRNA ligase, photosystem II extrinsic protein and ATP synthase increased by more than 27% in *M. aeruginosa* exposed to 0.0125 and 0.0500 mg/L Cd. The increased global effects on photosynthesis could help *M. aeruginosa* enhancing the carbon fixation and energy transfer, and thus promoting the synthesis of polysaccharides and colony formation. When the Cd contention was up to 0.0500 mg/L, the expression level of allophycocyanin subunit beta/alpha and universal stress protein UspA increased by 1.5 fold as compared to the control group, thus protecting DNA against oxidative damage (Bhat and Madyastha, 2001).



Enzymes involved in porphyrin and chlorophyll metabolism

**Figure 4.** Cd-induced changes in the expressions of enzymes involved in porphyrin and chlorophyll metabolism of *M. aeruginosa*



Enzymes involved in porphyrin and chlorophyll photosynthesis

**Figure 5.** Cd-induced changes in the expressions of enzymes involved in porphyrin and chlorophyll photosynthesis of *M. aeruginosa*

Consolidate all our findings about the effects of heavy metal ions on the *Microcystis* colony formation, we believe that *Microcystis* could bioaccumulate heavy metal ions, such as Cd, in natural waters, and appropriate heavy metal ions enriched in *Microcystis* cell could promote the synthesis and secretion of polysaccharide and MC in *M. aeruginosa* by upregulated polysaccharide and MC biosynthesis-related enzymes. Heavy metal ions could increase *Microcystis* colony formation in natural waters with the assistance of low concentration of MC, which may act as an important internal signal stimulator.

## Conclusions

Proper concentration of heavy metal ions could induce *Microcystis* colony formation. Heavy metal ions could increase *Microcystis* colony formation in natural waters with the assistance of low concentration of MC, which may act as an important internal signal stimulator. Further intensive study should be carried out to reveal the mechanism of synergistic reaction of MC and heavy metal ions.

**Acknowledgements.** This work is financially supported by National Natural Science Foundation of China (31772857) and Natural Science Foundation Grant of Tianjin (17JCYBJC29500, 18JCYBJC95900 and 16JCYBJC29900).

## REFERENCES

- [1] Alexova, R., Dang, T. C., Fujii, M., Raftery, M. J., Waite, T. D., Ferrari, B. C., Neilan, B. A. (2015): Specific global responses to N and Fe nutrition in toxic and non-toxic *Microcystis aeruginosa*. – *Environmental Microbiology* 18(2): 401-13.
- [2] Altschul, S. F., Gish, W. (1996): Local alignment statistics. – *Method in Enzymology* 266: 460-480.
- [3] Bhat, V. B., Madyastha, K. M. (2001): Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. – *Biochemical and Biophysical Research Communications* 285: 262-266.
- [4] Bi, X. D., Dai, W., Zhang, S. L., Xing, K. Z., Zhang, X. F. (2015): Accumulation and distribution characteristics of heavy metals in different size *Microcystis* colonies from natural waters. – *Fresenius Environmental Bulletin* 24: 773-779.
- [5] Bi, X. D., Yan, R., Li, F. X., Dai, W., Jiao, K. W., Zhou, Q. X., Liu, Q. (2016): Sequestration and distribution characteristics of Cd(II) by *Microcystis aeruginosa* and its role in colony formation. – *BioMed Research International Article* 9837598.
- [6] Bi, X. D., Dai, W., Zhang, S. L., Dong, S. J., Zhang, D. J. (2017): Effects of toxic *Microcystis* genotypes on natural colony formation and mechanism involved. – *Water Science and Technology* 76(3-4): 885-894.
- [7] Amorim, C. A., Ulisses, C., Moura, A. N. (2017): Biometric and physiological responses of *Egeria densa Planch.* cultivated with toxic and non-toxic strains of *Microcystis*. – *Aquatic Toxicology* 191: 201-208.
- [8] Gan, N. Q., Xiao, Y., Zhu, L., Wu, Z. X., Liu, J., Hu, C. L., Song, L. R. (2012): The role of microcystins in maintaining colonies of bloom-forming *Microcystis* spp.. – *Environmental Microbiology* 14(3): 730-742.
- [9] Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., Visser, P. M. (2018): Cyanobacterial blooms. – *Nature Reviews Microbiology* 16(8): 471-483.



- [10] Kanehisa, M., Goto, S., Hattori, M., Aoki-Kinoshita, K. F., Itoh, M., Kawashima, S., Katayama, T. (2006): From genomics to chemical genomics: new developments in KEGG. – *Nucleic Acids Research* 34(suppl 1): D354-D357.
- [11] Li, M., Zhu, W., Gao, L., Lu, L. (2013): Changes in extracellular polysaccharide content and morphology of *Microcystis aeruginosa* at different specific growth rates. – *Journal of Applied Phycology* 25(4): 1023-1030.
- [12] Liu, Y., Chen, S., Zhang, J., Gao, B. Y. (2016): Growth, microcystin-production and proteomic responses of *Microcystis aeruginosa* under long-term exposure to amoxicillin. – *Water Research* 93: 141-152.
- [13] Lyu, K., Meng, Q., Zhu, X., Dai, D., Zhang, L., Huang, Y., Yang, Z. (2016): Changes in iTRAQ-based proteomic profiling of the cladoceran *Daphnia magna* exposed to microcystin-producing and microcystin-free *Microcystis aeruginosa*. – *Environment Science Technology* 50(9): 4798-4807.
- [14] Magrane, M., UniProt Consortium (2011): UniProt Knowledgebase: a hub of integrated protein data. – *Database* bar009. DOI: 10.1093/database/bar009.
- [15] Olson, K. C., Chen, G., Lynch, C. J. (2013): Quantification of branched-chain keto acids in tissue by ultra fast liquid chromatography-mass spectrometry. – *Analytical Biochemistry* 439(2): 116-22.
- [16] O’Neil, J. M., Davis, T. W., Burford, M. A., Gobler, C. J. (2012): The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. – *Harmful Algae* 14: 313-334.
- [17] Omori, K., Datta, T., Amano, Y., Machida, M. (2019): Effects of different types of extracellular polysaccharides isolated from cyanobacterial blooms on the colony formation of unicellular *Microcystis aeruginosa*. – *Environmental Science and Pollution Research* 26(4): 3741-3750.
- [18] Sommaruga, R., Chen, Y. W., Liu, Z. W. (2008): Multiple strategies of bloom-forming *Microcystis* to minimize damage by solar ultraviolet radiation in surface waters. – *Microbial Ecology*, DOI: 10.1007/s00248-008-9425-4.
- [19] Shen, H., Song, L. R. (2007): Comparative studies on physiological responses to phosphorus in two phenotypes of bloom-forming *Microcystis*. – *Hydrobiologia* 592: 475-486.
- [20] Shen, H., Niu, Y., Xie, P., Tao, M., Yang, X. (2011): Morphological and physiological changes in *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. – *Freshwater Biology* 56: 1065-1080.
- [21] Tatusov, R. L., Fedorova, N. D., Jackson, J. D., Jacobs, A. R., Kiryutin, B., Koonin, E. V., Krylov, D. M. (2003): The COG database: an updated version includes eukaryotes. – *BMC bioinformatics* 4: 41.
- [22] Thurlow, L. R., Thomas, V. C., Hancock, L. E. (2009): Capsular polysaccharide production in *Enterococcus faecalis* and contribution of CpsF to capsule serospecificity. – *Journal of Bacteriology* 191(20): 6203-6210.
- [23] Tillett, D., Dittmann, E., Erhard, M., von Döhren, H., Börner, T., Neilan, B. A. (2000): Structural organization of microcystin biosynthesis in *Microcystis aeruginosa* PCC7806: an integrated peptide-polyketide synthetase system. – *Chemistry and Biology* 7(10): 753-764.
- [24] Wen, B., Zhou, R., Feng, Q., Wang, Q., Wang, J., Liu, S. (2014): IQuant: An automated pipeline for quantitative proteomics based upon isobaric tags. – *Proteomics* 14(20): 2280-2285.
- [25] Wiese, S., Reidegeld, K. A., Meyer, H. E., Warscheid, B. (2007): Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research. – *Proteomics* 7(6): 1004.
- [26] Wilmes, P., Bond, P. L. (2009): Microbial community proteomics: elucidating the catalysts and metabolic mechanisms that drive the Earth’s biogeochemical cycles. – *Current Opinion in Microbiology* 12(3): 310-317.

- [27] Xiao, M., Li, M., Reynolds, C. S. (2018): Colony formation in the cyanobacterium *Microcystis*. – Biological reviews 93(3): 1399-1420.
- [28] Xu, F., Zhu, W., Xiao, M., Li, M. (2016): Interspecific variation in extracellular polysaccharide content and colony formation of *Microcystis* spp. cultured under different light intensities and temperatures. – Journal of Applied Phycology 28: 1533-1541.
- [29] Yang, Z., Kong, F. X. (2012): Formation of large colonies: a defense mechanism of *Microcystis aeruginosa* under continuous grazing pressure by flagellate *Ochromonas* sp. – Journal of Limnology 171: 61-66.
- [30] Yang, Z., Kong, F. X., Shi, X. L., Xing, P., Zhang, M. (2007): Effects of Daphnia-associated infochemicals on the morphology, polysaccharides content and PSII-efficiency in *Scenedesmus obliquus*. – International Review of Hydrobiology 92: 618-625.
- [31] Yue, D., Peng, Y., Yin, Q., Xiao, L. (2015): Proteomic analysis of *Microcystis aeruginosa* in response to nitrogen and phosphorus starvation. – Journal of Applied Phycology 27: 1195-1204.
- [32] Zhang, M., Kong, F., Tan, X., Yang, Z., Cao, H., Xing, P. (2007): Biochemical, morphological, and genetic variations in *Microcystis aeruginosa* due to colony disaggregation. – World Journal of Microbiology and Biotechnology 23: 663-670.
- [33] Zhou, W., Juneau, P., Qiu, B. (2006): Growth and photosynthetic responses of the bloom-forming cyanobacterium *Microcystis aeruginosa* to elevated levels of cadmium. – Chemosphere 65(10): 1738-1746.
- [34] Zhu, W., Zhou, X. H., Chen, H. M., Gao, L., Xiao, M., Li, M. (2016): High nutrient concentration and temperature alleviated formation of large colonies of *Microcystis*: Evidence from field investigations and laboratory experiments. – Water Research 101(2016): 167-175.

## APPENDIX

Tables A1 and A4 are electronic appendices.

**Table A2.** The COG distribution of all identified proteins in *M. aeruginosa* FACHB-905

Abbreviation	Functional categories	Number
B	Chromatin structure and dynamics	1
C	Energy production and conversion	111
D	Cell cycle control, cell division, chromosome partitioning	21
E	Amino acid transport and metabolism	125
F	Nucleotide transport and metabolism	35
G	Carbohydrate transport and metabolism	83
H	Coenzyme transport and metabolism	70
I	Lipid transport and metabolism	34
J	Translation, ribosomal structure and biogenesis	104
K	Transcription	41
L	Replication, recombination and repair	38
M	Cell wall/membrane/envelope biogenesis	88
N	Cell motility	7
O	Posttranslational modification, protein turnover, chaperones	84
P	Inorganic ion transport and metabolism	61
Q	Secondary metabolites biosynthesis, transport and catabolism	46
R	General function prediction only	166
S	Function unknown	69
T	Signal transduction mechanisms	57
U	Intracellular trafficking, secretion, and vesicular transport	19
V	Defense mechanisms	15

**Table A3.** The KEGG distribution of all identified proteins in *M. aeruginosa* FACHB-905

No	Different proteins in annotated pathways	Number (percentage)
1	Metabolic pathways	411 (42.55%)
2	Biosynthesis of secondary metabolites	217 (22.46%)
3	Biosynthesis of antibiotics	136 (14.08%)
4	Microbial metabolism in diverse environments	118 (12.22%)
5	Biosynthesis of amino acids	91 (9.42%)
6	Carbon metabolism	73 (7.56%)
7	Ribosome	43 (4.45%)
8	Purine metabolism	41 (4.24%)
9	Amino sugar and nucleotide sugar metabolism	31 (3.21%)
10	Photosynthesis	31 (3.21%)
11	ABC transporters	30 (3.11%)
12	Oxidative phosphorylation	29 (3%)
13	Glycolysis/Gluconeogenesis	29 (3%)
14	Porphyrin and chlorophyll metabolism	27 (2.8%)
15	Pyruvate metabolism	26 (2.69%)
16	2-Oxocarboxylic acid metabolism	25 (2.59%)
17	Two-component system	24 (2.48%)
18	Starch and sucrose metabolism	24 (2.48%)
19	Aminoacyl-tRNA biosynthesis	24 (2.48%)
20	Carbon fixation pathways in prokaryotes	23 (2.38%)
21	Methane metabolism	22 (2.28%)
22	Glyoxylate and dicarboxylate metabolism	22 (2.28%)
23	Alanine, aspartate and glutamate metabolism	22 (2.28%)
24	Pyrimidine metabolism	22 (2.28%)
25	Glycine, serine and threonine metabolism	21 (2.17%)
26	Fructose and mannose metabolism	21 (2.17%)
27	RNA degradation	21 (2.17%)
28	Cysteine and methionine metabolism	20 (2.07%)
29	Pentose phosphate pathway	19 (1.97%)
30	Carbon fixation in photosynthetic organisms	19 (1.97%)
31	Photosynthesis - antenna proteins	18 (1.86%)
32	Citrate cycle (TCA cycle)	17 (1.76%)
33	Fatty acid metabolism	17 (1.76%)
34	Fatty acid biosynthesis	16 (1.66%)
35	Nitrogen metabolism	16 (1.66%)
36	Glutathione metabolism	14 (1.45%)
37	Valine, leucine and isoleucine biosynthesis	14 (1.45%)
38	Arginine biosynthesis	13 (1.35%)
39	Propanoate metabolism	13 (1.35%)
40	Lysine biosynthesis	12 (1.24%)
41	Phenylalanine, tyrosine and tryptophan biosynthesis	12 (1.24%)
42	Butanoate metabolism	12 (1.24%)
43	Arginine and proline metabolism	12 (1.24%)
44	Histidine metabolism	11 (1.14%)
45	Nonribosomal peptide structures	11 (1.14%)
46	Sulfur metabolism	10 (1.04%)
47	Streptomycin biosynthesis	10 (1.04%)
48	C5-Branched dibasic acid metabolism	10 (1.04%)
49	Terpenoid backbone biosynthesis	10 (1.04%)
50	Ubiquinone and other terpenoid-quinone biosynthesis	10 (1.04%)
51	Nicotinate and nicotinamide metabolism	10 (1.04%)
52	Biotin metabolism	10 (1.04%)
53	Galactose metabolism	9 (0.93%)

54	Peptidoglycan biosynthesis	9 (0.93%)
55	Mismatch repair	9 (0.93%)
56	Pantothenate and CoA biosynthesis	9 (0.93%)
57	One carbon pool by folate	9 (0.93%)
58	Peroxisome	8 (0.83%)
59	Central carbon metabolism in cancer	8 (0.83%)
60	Cell cycle - Caulobacter	8 (0.83%)
61	Bacterial secretion system	8 (0.83%)
62	Tyrosine metabolism	8 (0.83%)
63	Homologous recombination	8 (0.83%)
64	Tuberculosis	8 (0.83%)
65	DNA replication	8 (0.83%)
66	Protein export	7 (0.72%)
67	Glucagon signaling pathway	7 (0.72%)
68	Nucleotide excision repair	7 (0.72%)
69	Cationic antimicrobial peptide (CAMP) resistance	7 (0.72%)
70	Carotenoid biosynthesis	7 (0.72%)
71	Biosynthesis of unsaturated fatty acids	6 (0.62%)
72	Legionellosis	6 (0.62%)
73	Fatty acid degradation	6 (0.62%)
74	Folate biosynthesis	6 (0.62%)
75	Thiamine metabolism	6 (0.62%)
76	Degradation of aromatic compounds	5 (0.52%)
77	Drug metabolism - other enzymes	5 (0.52%)
78	Selenocompound metabolism	5 (0.52%)
79	Monobactam biosynthesis	5 (0.52%)
80	Valine, leucine and isoleucine degradation	5 (0.52%)
81	Base excision repair	5 (0.52%)
82	Drug metabolism - cytochrome P450	5 (0.52%)
83	Inositol phosphate metabolism	5 (0.52%)
84	Phenylalanine metabolism	5 (0.52%)
85	Cyanoamino acid metabolism	5 (0.52%)
86	Vitamin B6 metabolism	5 (0.52%)
87	Salmonella infection	5 (0.52%)
88	Type I polyketide structures	4 (0.41%)
89	Protein processing in endoplasmic reticulum	4 (0.41%)
90	Chemical carcinogenesis	4 (0.41%)
91	Metabolism of xenobiotics by cytochrome P450	4 (0.41%)
92	Aminobenzoate degradation	4 (0.41%)
93	Tryptophan metabolism	4 (0.41%)
94	Polyketide sugar unit biosynthesis	4 (0.41%)
95	Glycerophospholipid metabolism	4 (0.41%)
96	Sulfur relay system	4 (0.41%)
97	Riboflavin metabolism	4 (0.41%)
98	Pentose and glucuronate interconversions	4 (0.41%)
99	Insulin signaling pathway	4 (0.41%)
100	Chloroalkane and chloroalkene degradation	4 (0.41%)
101	HIF-1 signaling pathway	4 (0.41%)
102	GABAergic synapse	4 (0.41%)
103	Biosynthesis of ansamycins	4 (0.41%)
104	Glycerolipid metabolism	4 (0.41%)
105	RNA polymerase	4 (0.41%)
106	Tetracycline biosynthesis	4 (0.41%)
107	Lipopolysaccharide biosynthesis	3 (0.31%)
108	AMPK signaling pathway	3 (0.31%)

109	Pathways in cancer	3 (0.31%)
110	Huntington's disease	3 (0.31%)
111	Viral carcinogenesis	3 (0.31%)
112	Toluene degradation	3 (0.31%)
113	Type II diabetes mellitus	3 (0.31%)
114	Biosynthesis of siderophore group nonribosomal peptides	3 (0.31%)
115	Chlorocyclohexane and chlorobenzene degradation	3 (0.31%)
116	Fluorobenzoate degradation	2 (0.21%)
117	PPAR signaling pathway	2 (0.21%)
118	Mineral absorption	2 (0.21%)
119	Limonene and pinene degradation	2 (0.21%)
120	N-Glycan biosynthesis	2 (0.21%)
121	Taurine and hypotaurine metabolism	2 (0.21%)
122	Synaptic vesicle cycle	2 (0.21%)
123	Sphingolipid metabolism	2 (0.21%)
124	Isoquinoline alkaloid biosynthesis	2 (0.21%)
125	Stilbenoid, diarylheptanoid and gingerol biosynthesis	2 (0.21%)
126	Phosphatidylinositol signaling system	2 (0.21%)
127	Insulin resistance	2 (0.21%)
128	Naphthalene degradation	2 (0.21%)
129	Benzoate degradation	2 (0.21%)
130	Atrazine degradation	2 (0.21%)
131	FoxO signaling pathway	2 (0.21%)
132	Lysine degradation	2 (0.21%)
133	Tropane, piperidine and pyridine alkaloid biosynthesis	2 (0.21%)
134	RNA transport	2 (0.21%)
135	Bisphenol degradation	2 (0.21%)
136	Adipocytokine signaling pathway	2 (0.21%)
137	Alzheimer's disease	2 (0.21%)
138	Phenylpropanoid biosynthesis	2 (0.21%)
139	Vasopressin-regulated water reabsorption	2 (0.21%)
140	Meiosis - yeast	2 (0.21%)
141	Type I diabetes mellitus	2 (0.21%)
142	Glutamatergic synapse	2 (0.21%)
143	Novobiocin biosynthesis	2 (0.21%)
144	Vancomycin resistance	2 (0.21%)
145	Endocytosis	1 (0.1%)
146	Flavonoid biosynthesis	1 (0.1%)
147	Isoflavonoid biosynthesis	1 (0.1%)
148	Renal cell carcinoma	1 (0.1%)
149	beta-Lactam resistance	1 (0.1%)
150	Polycyclic aromatic hydrocarbon degradation	1 (0.1%)
151	Linoleic acid metabolism	1 (0.1%)
152	Nitrotoluene degradation	1 (0.1%)
153	Influenza A	1 (0.1%)
154	Hepatitis B	1 (0.1%)
155	NOD-like receptor signaling pathway	1 (0.1%)
156	mRNA surveillance pathway	1 (0.1%)
157	PI3K-Akt signaling pathway	1 (0.1%)
158	Cocaine addiction	1 (0.1%)
159	Thyroid hormone synthesis	1 (0.1%)
160	Biosynthesis of type II polyketide products	1 (0.1%)
161	Toxoplasmosis	1 (0.1%)
162	Styrene degradation	1 (0.1%)
163	D-Glutamine and D-glutamate metabolism	1 (0.1%)

164	p53 signaling pathway	1 (0.1%)
165	Plant-pathogen interaction	1 (0.1%)
166	beta-Alanine metabolism	1 (0.1%)
167	Retinol metabolism	1 (0.1%)
168	Ethylbenzene degradation	1 (0.1%)
169	D-Alanine metabolism	1 (0.1%)
170	Parkinson's disease	1 (0.1%)
171	Non-alcoholic fatty liver disease (NAFLD)	1 (0.1%)
172	Prostate cancer	1 (0.1%)
173	Viral myocarditis	1 (0.1%)
174	Biosynthesis of 12-, 14- and 16-membered macrolides	1 (0.1%)
175	Colorectal cancer	1 (0.1%)
176	Alcoholism	1 (0.1%)
177	Amphetamine addiction	1 (0.1%)
178	Serotonergic synapse	1 (0.1%)
179	Steroid hormone biosynthesis	1 (0.1%)
180	Phosphonate and phosphinate metabolism	1 (0.1%)
181	Other glycan degradation	1 (0.1%)
182	Glycosaminoglycan biosynthesis - chondroitin sulfate/dermatan sulfate	1 (0.1%)
183	Ascorbate and aldarate metabolism	1 (0.1%)
184	Epithelial cell signaling in <i>Helicobacter pylori</i> infection	1 (0.1%)
185	Small cell lung cancer	1 (0.1%)
186	Progesterone-mediated oocyte maturation	1 (0.1%)
187	Synthesis and degradation of ketone bodies	1 (0.1%)
188	Apoptosis	1 (0.1%)
189	Amyotrophic lateral sclerosis (ALS)	1 (0.1%)
190	Renin-angiotensin system	1 (0.1%)
191	Carbapenem biosynthesis	1 (0.1%)
192	Herpes simplex infection	1 (0.1%)
193	Antigen processing and presentation	1 (0.1%)
194	Dopaminergic synapse	1 (0.1%)
195	Estrogen signaling pathway	1 (0.1%)
196	Butirosin and neomycin biosynthesis	1 (0.1%)
197	Biosynthesis of vancomycin group antibiotics	1 (0.1%)