Journal of Hazardous Materials 161 (2009) 730–736

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Effects of amino acids on microcystin production of the *Microcystis aeruginosa*

Ruihua Dai[a,b], Huijuan Liu[a], Jiuhui Qu[a,*], Xu Zhao[a], Yining Hou[a,b]

[a] State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, China

ARTICLE INFO

Article history:
Received 18 November 2007
Received in revised form 14 March 2008
Accepted 5 April 2008
Available online 11 April 2008

Keywords:
Microcystis aeruginosa
Growth
Production
Microcystin-LR
Dissolved free amino acids

ABSTRACT

A *Microcystis aeruginosa* which produced high content of microcystin-LR (MC-LR) but no microcystin-RR (MC-RR) was isolated from Dianchi Lake in China. In the molecular structure of MC-LR, glutamic acid, aspartic acid, leucine, alanine and arginine are the constitutional components which are abundant in natural water. In this paper, effects of six amino acids at their natural concentrations on the growth of the *M. aeruginosa* and the microcystin (MC) production were studied in batch culture. *M. aeruginosa* could assimilate alanine, leucine and arginine as sole nitrogen sources for growth and MC production. However, glutamic acid, aspartic acid and lysine could not be assimilated quickly, although they could pass the cell membrane and enter into the cell rapidly. Our experiment demonstrated that the possible reason of such phenomenon was that different amino acids had different effects on the process of metabolism through the free dissolved amino acids within the cells.

1. Introduction

Toxic cyanobacterial algal blooms frequently occur in eutrophic lakes, ponds and reservoirs all over the world [1–3]. It is well known that causative *cyanobacteria* (*Microcystis aeruginosa*, *Anabaena*, and *Oscillatoria*) produce microcystins (MCs) or other cyclic hepatotoxins [4,5]. Among the toxic cyanobacteria, *M. aeruginosa* is commonly observed in highly eutrophic lakes [6,7]. The MCs produced by *M. aeruginosa* have been the cause of several poisonings of domestic animals and wildlife around the world, and they also pose a health hazard for humans through the use of water for drinking and recreation [3,8].

Certain environmental factors, such as nitrogen, phosphorus, pH and temperature, seem to affect the production of MCs and extensively to be studied in both batch and continuous cultures [9–12]. It is well known that nitrogen is not only a main nutrient for cyanobacterial growth but also a key element in the molecular of MCs. Therefore, many scientists have focused on the effects of nitrogen, especially dissolved inorganic forms of nitrogen (DIN) on MCs production using the *M. aeruginosa* species [11,13–16].

In summer-temperature waters, DIN has declined to minima while dissolved organic nitrogen (DON) rise to a maximum [17]. A change in the nutrient regime may enable *M. aeruginosa* capable of utilizing organic forms of nitrogen to become dominant [18]. Variable nitrogenous forms are in eutrophic water; however, one of the most available forms of DON are dissolved free amino acids (DFAAs), such as alanine, arginine, leucine, lysine, glutamic acid, aspartic acid and so on. It is possible that when nitrogen-deprived, *M. aeruginosa* may also develop the ability to take up DFAAs and utilize them to synthesize the MCs [19]. However, comparative studies on MCs production by *M. aeruginosa* have been limited to investigate the effects of inorganic nitrogen. The role of dissolved organic nitrogen (DON) has not been paid much attention.

Microcystins, a family of hepatotoxins produced by *M. aeruginosa*, are monocyclic heptapeptides constituted by five constant and two variable amino acids. When the variable amino acids are leucine and arginine, the MC is indicated as microcystin-LR (MC-LR) [20]. As we all known, the molecular structure contains alanine, leucine, arginine, glutamic acid and aspartic acid as the constitutional components. These amino acids are the most abundant compounds in natural water [21,22]. Therefore, they may be responsible for the growth and toxin production of *M. aeruginosa*.

Xu and Zeng [23] pointed out that *M. aeruginosa* could take up amino acids and utilize them to synthesize protein. Yan et al. [19] used leucine and arginine as nitrogen source which described leucine and arginine both inhibited the MC production, although leucine slightly promoted the growth; however, in such growth experiments nitrogenous compounds are usually 1000× more concentrated than in natural waters (mM rather than μM). The ability of *M. aeruginosa* to use amino acids as nitrogen sources at high concentrations does not necessarily provide any insight into their potential significance at low concentrations. Moreover, they could

* Corresponding author at: State Key Laboratory of Environmental Aquatic Chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P.O. Box 2871, Beijing 100085, China. Tel.: +86 10 62849151; fax: +86 10 62849160.
E-mail address: jhqu@rcees.ac.cn (J. Qu).

0304-3894/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2008.04.015

not explain the reason of different response with different amino acids further. In the present study, the aims were to investigate the ability of *M. aeruginosa* to utilize DFAAs as nitrogen sources at natural concentrations, and to determine whether DFAAs can influence its growth and MC production in axenic culture.

2. Materials and methods

2.1. M. aeruginosa and cultures

An axenic strain of *M. aeruginosa* which was isolated from Dianchi Lake in China was obtained from Institute of Hydrobiology, Chinese Academy of Science. The *M. aeruginosa* produced microcystin-LR (MC-LR) only [24].

M. aeruginosa was maintained in BG11 medium. Initial pH of the medium was adjusted to 8.5 using 1.0 mol/L hydrochloric acid and sodium hydroxide solutions. The medium and all experimental utensils were kept at 121 °C for 30 min in order to sterilize the bacteria. Cultures in exponential phase were concentrated by centrifugation and washed three times with sterile distilled water and then inoculated in the same growth medium, but without a nitrogen source for a week to exhaust the nitrogen in the cells. The *M. aeruginosa* were then added to a series of 2.5 L flat-bottomed bottles which contained 2 L medium and supplemented with 100 μmol/L of nitrogen of one of the following: glutamic acid, aspartic acid, leucine, alanine, lysine and arginine. Control bottles were incubated with the same algal inoculums in NO_3–N medium.

All samples were run in triplicate bottles at 26 °C and a light intensity of 400–650 lx with a 12 h photoperiod. Subsamples were taken through sterilized tube at predetermined intervals after inoculation to count the cell number, extract the MC and amino acids in cells during the incubation period.

2.2. Measurement of cell number

After straining with Lugol's Iodine solution, the population of *M. aeruginosa* cells was twice counted under a microscope using a hemocytometer. Cell counts were performed to a minimum precision of 20%, or the third cell count was needed. At the same time, the medium was inspected to certify whether *M. aeruginosa* was contaminated by extraneous bacterium.

2.3. Determination of MC within the cells

50 mL cyanobacterial culture solution was taken at 12 h, 24 h, 48 h, 96 h, 120 h, 180 h and 240 h after inoculation. The samples were centrifuged at 8000 rpm for 10 min and then the supernatant was discarded. The cell pellet was frozen under −20 °C over 24 h. 20 mL 50% methanol–water solution was added to the cell pellet to extract MC within cyanobacterial cells. The extraction solution was disrupted using Ultrasonic Cell Disruptor (JY92-2D, SCIENTZ, China) for 30 min and then was put in boiling water for 20 min to extract MC. The solution was centrifuged at 8000 rpm for 10 min and the supernatant was filtered through 0.22 μm membranes. The filtration was enriched to 1 mL by mild nitrogen gas with Nitrogen Evaporator (KL512, Kanglin Co., China) and measured for MC on HPLC. The MC content was calculated from the ratio between the concentration of MC in the extraction solution (μg/L) and the dry weight in the subsamples (mg/mL). The data presented here was the average values of three parallel samples with the standard deviation.

Standard microcystin-RR (MC-RR) and microcystin-LR (MC-LR) bought from sigma (98% purity) and samples of MC-RR and MC-LR were analyzed by HPLC (Hitachi L-2000) with a reverse ZORBAX SB-C18 column (4.6 mm × 250 mm, Agilent Co., USA) and a Diode Array Detector at 238 nm. The mobile phase was 35% (v/v) acetonitrile–water solution containing 0.05% (v/v) of frozen acetic acid. The flow rate was 1.0 mL/min and the injection amount was 20 μL.

2.4. Determination of amino acids within the cells and in the culture

The contents of amino acids in culture and uptake rates were measured at 12 h, 24 h, 48 h and 96 h during growth on batch culture. For each measurement, 10 mL cultures were harvested to centrifuge the cells. The concentration of amino acids was analyzed in the supernatant. Uptake rates were computed from the decrease per hours in the supernatant samples, and are presented as V (nitrogen taken up h^{-1} $cell^{-1}$): $V = \frac{\mu mol\, N_i - \mu mol\, N_f}{l \times m}$ where N_i and N_f were the initial and final concentration of amino acids, respectively, l was the interval time, and m was the number of *M. aeruginosa* cells.

Intracellular amino acids were extracted as following procedure. At 48 h, 100 mL cyanobacterial culture solution was centrifuged at 8000 rpm for 10 min and then the supernatant was discarded. After adding some distilled water to resuspend the pellets, the solution was centrifuged and the supernatant was also discarded. This approach was repeated for three times in order to rinse the amino acids out of the pellets. 2 mL 0.1 mol/L hydrochloric acid was added into the pellets and the solution was disrupted using Ultrasonic Cell Disruptor (JY92-2D, SCIENTZ, China) for 30 min to extract the amino acids. After that, the solution was centrifuged for 10 min. The supernatant was poured into centrifugal tube. This process was repeated two times and all the supernatant were mixed in the centrifugal tube. Following that, 1 mL 9% 5-sulfosalicylic acid dihydrate was added into the centrifugal tube to deposit the protein (in ice-jar for 30 min), and then the solution was centrifuged at 8000 rpm for 30 min in 4 °C. At last, the supernatant was put into the centrifugal tube with scale and was diluted to 10 mL with 0.1 mol/L hydrochloric acid. The amino acids were measured with amino acid analyzer (SYKAM, Germany).

3. Results

3.1. Effects of DFAAs on the growth of M. aeruginosa

In summer, when DIN was exhaust, *M. aeruginosa* might assimilate DFAAs to support their growth. As shown in Fig. 1, different amino acids had different effects on the growth of *M. aeruginosa*. It was shown that *M. aeruginosa* could grow well in the medium containing alanine, arginine, and leucine, respectively, and could not grow in lysine (Fig. 1(b)). However, at the presence of glutamic acid and aspartic acid, *M. aeruginosa* grew faster than that of other amino acid until 96 h and then became yellow soon. Fig. 1 indicated that the growth of *M. aeruginosa* in the medium containing arginine, leucine and alanine reached exponential phases at the 120 h and late-exponential phases at the 240 h and their growth was a typical example of batch culture growth. Cells of *Microcystis areugniosa* grown on alanine, arginine and leucine attained maximum numbers of 15.78×10^6, 13.84×10^6 and 13.18×10^6 cells/mL, respectively. Correspondingly at the presence of aspartic acid and glutamic acid the maximum cell numbers was up to 10.9×10^6, 9.45×10^6 cells/mL at 96 h, respectively, and exceeded the cell number of alanine at the same time (Fig. 1). However, the growth ceased after 96 h and the appearance of culture became yellow and turbid. Lysine in the medium could not sustain the growth of *M. aeruginosa* at all and the biomass was lower than that of blank (Fig. 1(b)).

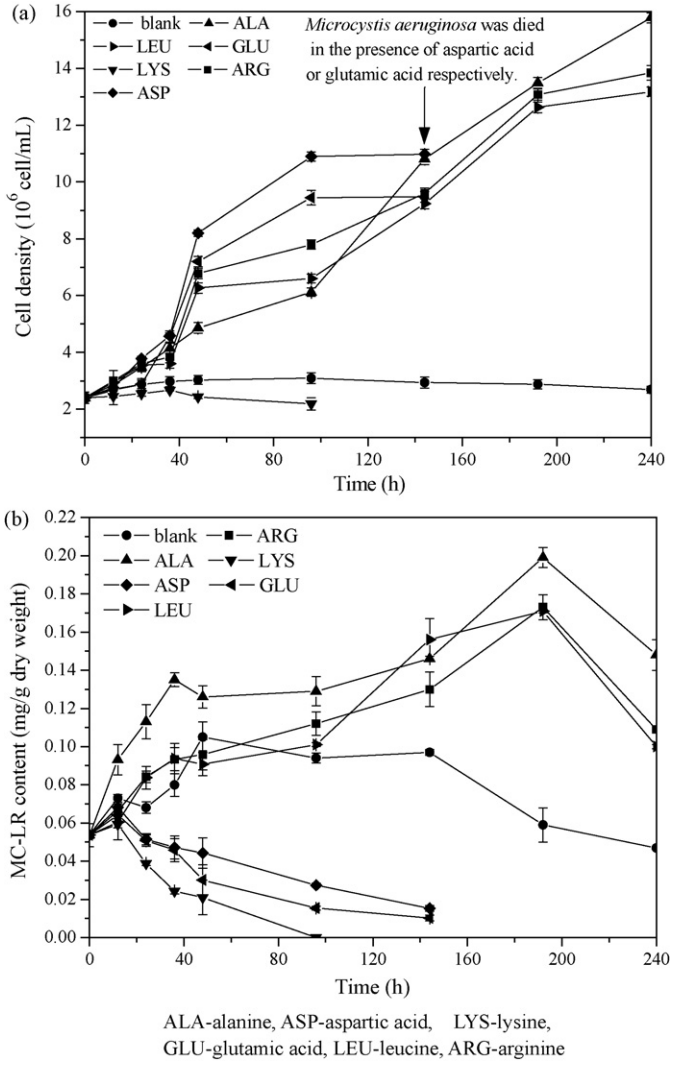

Fig. 1. Effects of amino acids on the growth and the production of MC-LR of *Microcystis aeruginosa*. (a) Alanine, leucine and glutamic acid; (b) Arginine, aspartic acid and lysine.

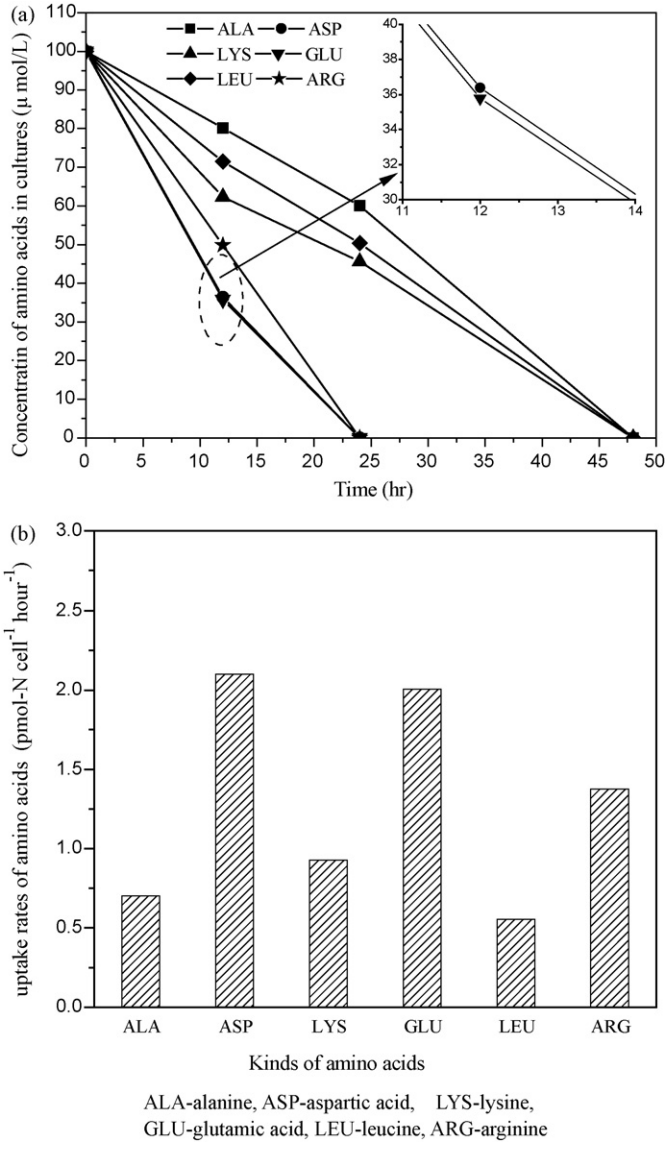

Fig. 2. The graph of *M. aeruginosa* assimilation amino acid in axenic culture. (a) The concentration of amino acids in medium at different time; (b) the uptake rates of amino acids at 12 h.

3.2. Effects of DFAAs on the production of MC

The above study demonstrated that *M. aeruginosa* could take up alanine, leucine and arginine, and could not absorb glutamic acid, aspartic acid and lysine. However, whether those amino acids could promote microcystins production was more important. Fig. 1 showed the microcystin content with different amino acids as nitrogen source. It was noticeable that the MC content at the presence of alanine, arginine and leucine gradually enhanced and obtained the maximum at 192 h from late-lag phase to exponential phases; moreover, the MC content declined at the late-exponential phases as shown in Fig. 1(a) and (b). These results agreed with literatures [14,15,19]. Fig. 1(a) and (b) also showed alanine promoted higher production of MC-LR than that of arginine and leucine. The maximum content of MC-LR in alanine could reach 0.199 mg/g dry weight at 192 h. The MC production of leucine and arginine ranked the second and the third places with 0.171 mg/g dry weight and 0.173 mg/g dry weight at 192 h, respectively.

As shown in Fig. 1, glutamic acid and aspartic acid, which hugely promoted the growth of *M. aeruginosa* at the beginning of incubation, did not promote the production of MC-LR. Their maximum MC production was 0.064 and 0.0682 mg/g dry weight at 12 h, respectively, and less than that of blank (0.105 mg/g dry weight). The medium containing lysine did not increase the growth and the production of MC-LR (Fig. 1(b)). Its MC production was always lower than that of blank in the whole batch experiments.

3.3. Uptake of amino acids of M. aeruginosa

In order to know whether those amino acids were utilized and which amino acids were assimilated quickly, the concentrations of amino acids in cultures were studied. Fig. 2(a) showed the concentration of amino acids in the cultures at 12 h, 24 h and 48 h and Fig. 2(b) was the uptake rates at 12 h in axenic cultures. The ability of *M. aeruginosa* to accumulate different amino acids from low concentrations was different. When cells were transferred to growth medium, they took up the amino acids in the medium immediately. *M. aeruginosa* showed very rapid uptake of aspartic acid and glutamic acid, while alanine and leucine were taken up slowly as shown in Fig. 2(a). During the first 12 h, the uptake rates of *M. aeruginosa* at the presence of aspartic acid and glutamic

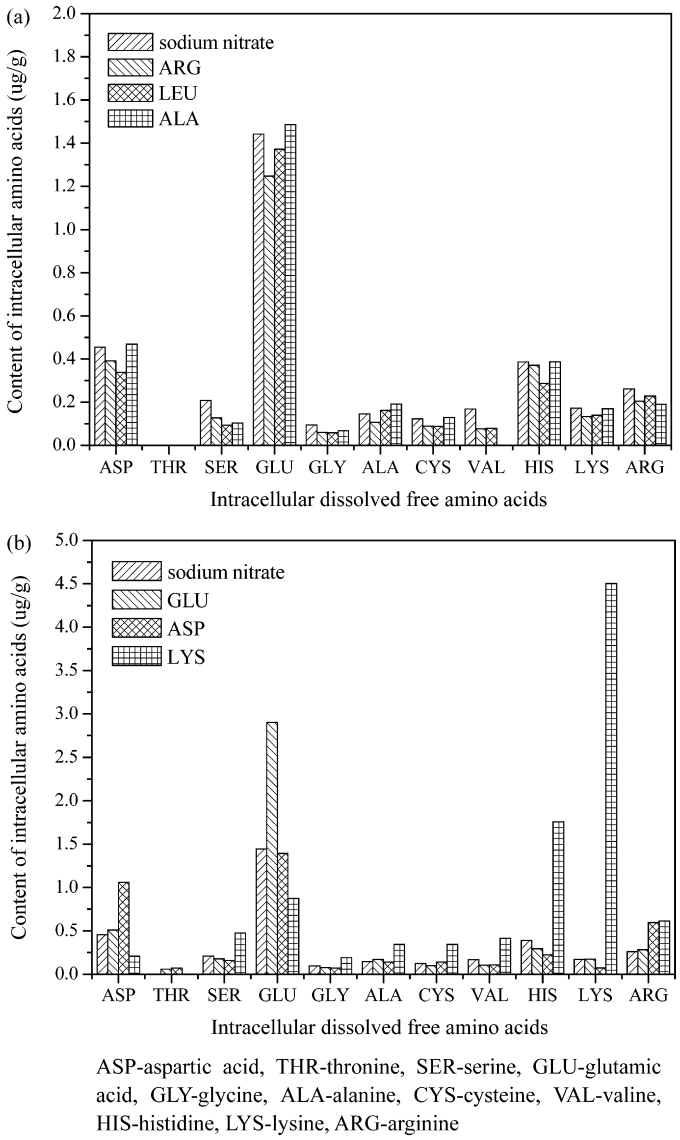

Fig. 3. The contents of intracellular dissolved free amino acids with different amino acids as nitrogen source at 48 h in axenic culture.

acid were up to 2.10 and 2.01 pmol N cell^{-1} h^{-1}, respectively. The uptake rates at the presence of lysine and arginine were 0.93 and 1.37 pmol N cell^{-1} h^{-1}, respectively. The uptake rates of *M. aeruginosa* with alanine and leucine as nitrogen source were lower. At 24 h, glutamic acid, arginine and aspartic acid all disappeared from the medium. However, until 48 h, alanine, lysine and leucine did not disappear.

3.4. Intracellular dissolved free amino acids in the cells

Fig. 3(a) and (b) showed that the intracellular dissolved free amino acids in cells with sodium nitrate, alaine, leucine, arginine, glutamic acid, aspartic acid and lysine. Fig. 3 demonstrated that in the normal cells of *M. aeruginosa* glutamic acid and aspartic acid are the most abundant dissolved free amino acids, especially the glutamic acid. Its concentration is up to about 1.44 μg/g. When gutamic acid or aspartic acid was used as nitrogen source, dissolved free glutamic acid or aspartic acid within the cells were largely accumulated and their concentrations were higher than that of normal cells (NO_3–N as nitrogen source). Fig. 3(b) also showed that the concentrations of dissolved free lysine and histidine in cells were much higher, while the concentrations of glutamic acid and aspartic acid were lower than that of normal cells.

3.5. SEM images

The SEM images of *M. aeruginosa* in the presence of alanine, glutamic acid and lysine were shown in the Fig. 4(a), (b) and (c), respectively. It is obvious that normal cells (Fig. 4(a)) are round and plump and have a spherical shape with a smooth exterior. In comparing with the normal *M. aeruginosa* cells, the cells in the presence of glutamic acid or aspartic acid had some changes. The cells were distorted from their normal spherical shape and appeared flattened. Moreover, some of cells were cracked and their inclusion leaked out. At the presence of lysine, most of the cells cracked severely and much inclusion was released.

The MC-LR content in glutamic acid, lysine and aspartic acid culture medium was measured, but it was under the detection limit.

4. Discussion

Our results clearly demonstrated that alanine, leucine and arginine could be used as sole nitrogen source to support the growth of *M. aeruginosa* and promote the MC production. *M. aeruginosa* could not utilize amino-N of glutamic acid, aspartic acid and lysine to support the natural and sustaining growth, even though it can take up those dissolved free amino acids rapidly. Especially, *M. aeruginosa* absolutely failed to grow on lysine. Therefore, glutamic acid, aspartic acid and lysine could not stimulate *M. aeruginosa* to produce MC.

Dissolved free amino acids could provide an additional source of nitrogen for the growth of microalgae [17]. In the present study, it is interesting to find that different amino acids have different effects on the algal growth and MC production. Ahluwalia [25] described that growth inhibition caused by amino acids could be due to the feedback inhibition of biosynthetic enzymes. Different responses of some amino acids are also attributed to changes in permeability and uptake process [25]. In this experiment, the axenic culture was required to ensure that amino-N is being used directly by the *M. aeruginosa* rather than via bacterial regeneration of ammonium. No extraneous bacterium was observed during all the culture period. The uptake processes of microalgae were reported in many studies. *Amphidinium* spp. has shown to assimilate amino-N by the use of cell-surface amino oxidase activity [26]. *Chlorella* was reported to possess both general and specific amino acid transport system [27]. The uptake of amino acid in *M. aeruginosa* may be by the special transport system, the amino oxidase or other unknown process. The reasons of different amino acids having different effects on the algal and MC production may be that different amino acids had different responses to normal physiological metabolism, biosynthetic enzymes, uptake process and so on. We discussed the reason from the aspect of normal physiological metabolism through the intracellular dissolved amino acids and the exterior shape (SEM images).

Turner [28] reported that algae could directly take up amino acid from medium if the algae just needed the amino acid. With respect to our experiments, as shown in Figs. 2 and 3(a), it is apparent that *M. aeruginosa* could take up alanine, leucine and arginine and then assimilate them rapidly and directly. When alanine, leucine and arginine were as sole nitrogen source in the medium, *M. aeruginosa* decompounded these amino acids by decarboxylation, deamination or transamination to absorb them and had them support its growth. During these processes, *M. aeruginosa* could assimilate this

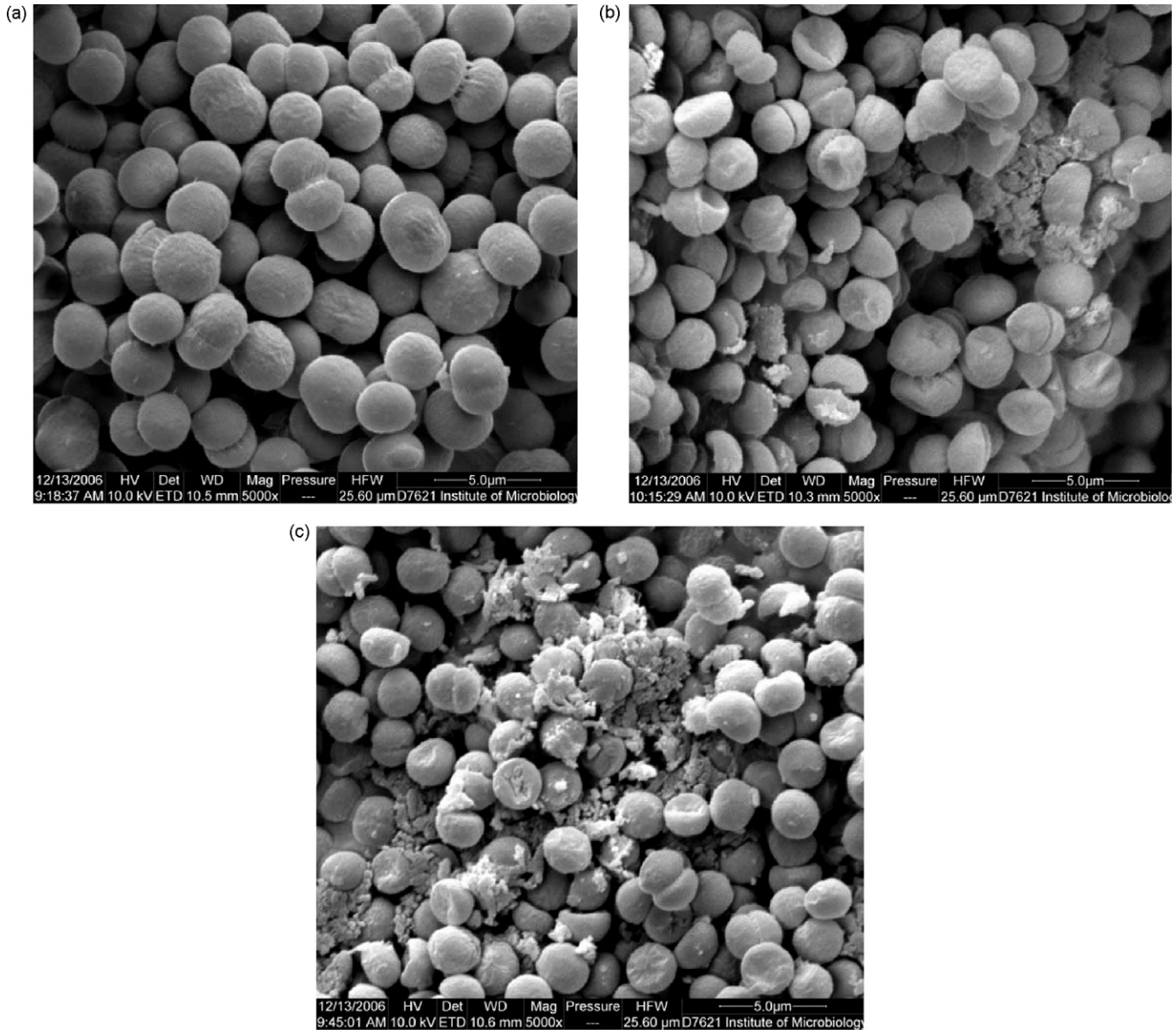

Fig. 4. The SEM images of *M. aeruginosa* at the presence of different amino acids. (a) alanine (5000×); (b) glutamic acid (5000×); (c) lysine (5000×).

amino-N which was similar with the normal process. It is clearly evident from the data of Fig. 3(a).

The SEM image, as shown in Fig. 4(a), demonstrated that cells of *M. aeruginosa* in presence of alanine were round and plump as the normal cells. Fig. 3(a) clarified the kinds and concentrations of intracellular dissolved free amino acids in the cells with alanine, leucine and arginine as nitrogen source were similar with that of normal cells ($NaNO_3$ as nitrogen source). This result agreed with Fig. 4(a) and they both showed that extra alanine, leucine, arginine in the growth culture could support the normal physiological metabolism.

Alanine, leucine and arginine are the constitutional components in the molecular structures of MC and promoted MC production and growth. It is possible that these amino acids were directly utilized to synthesize MC-LR or participated in the process of MC synthesis. If these amino acids exist in natural water, they may stimulate the water bloom of *M. aeruginosa* and give rise to the MC production. Yan et al. [19] described that leucine and arginine all inhibited the MC production, although leucine slightly promoted the growth. The different concentration of amino acid which we used (100 μM) and Yan (mM rather than μM) used may lead to the conflicting result.

As shown in Fig. 3(a), it demonstrated that in the normal cell of *M. aeruginosa* glutamic acid and aspartic acid are the most abundant dissolved free amino acids, especially the glutamic acid. This phenomenon may testify that the theory that synthesis of other amino acids is through the activities of glutamic acid and aspartic acid [29].

During the metabolism of amino acid, glutamic acid and aspartic acid are important to nitrogen assimilation, excretion and transfer. Nitrogen is transferred from glutamate and aspartate via transamination to valine, isoleucine, leucine, tyrosine, phenylalanine and other amino acids [29]. When glutamic acid or aspartic acid is used as sole nitrogen source, no nitrogen is needed to be transferred. Although glutamic acid or aspartic acid could entered the cells as shown in Fig. 2, they could not be assimilated and could accumulate in the cells. Fig. 3(b) showed dissolved free glutamic acid

or aspartic acid within the cells were largely accumulated when using glutamic acid or aspartic acid as nitrogen source and their concentrations were higher than that of normal cells (NO_3–N as nitrogen source). This accumulation interfered with the normal physiological metabolism of the *M. aeruginosa*; therefore, glutamic acid and aspartic acid in the medium made the *M. aeruginosa* grow very rapidly and soon become yellow. This result was testified by Fig. 4(b). The cells were intimidated by glutamic acid or aspartic acid and were distorted from their normal spherical shape and appeared flattened; moreover, some of cells were cracked and the inclusion leaked out. Glutamic acid or aspartic acid disordered the normal metabolism and also the pathway of MC synthesis. Therefore, the MC production was low.

As shown in Fig. 2, lysine could be taken up rapidly, but it could not support the growth of *M. aeruginosa* and did not promote the MC production. Fig. 3(b) testified that the concentration of dissolved free lysine and histidine in cells was much higher than that of normal cells. Lysine entered the cells of *M. aeruginosa* quickly, but it could not be decomposed and utilized effectively. Lysine in the cells inhibited the normal transmission of amino acids and other metabolism.

Fig. 4(c) showed most of the cells cracked severely and much inclusion was released. Lysine may be responsible for causing severe damage to the cell wall in *M. aeruginosa* and it further inhibited new cell wall synthesis, enzymatics, reactions, or photosynthesis. The lack of membrane bound organelles may allow for a greater and more rapid disruption of photosynthesis. Therefore, *M. aeruginosa* stopped growth at the presence of lysine and stopped to produce MC also.

Hehmann et al. [30] reported that lysine was used to control the water bloom containing *M. aeruginosa*. *M. aeruginosa* could be inhibited by lysine at concentration between 0.6 and 5.0 mg/L. In a similar report, Yamamoto et al. [31] also described that cell walls of *M. aeruginosa* disappeared after lysine treatment. Our results are consistent with them.

In theory, any nitrogenous compound which passes the plasma membrane and enters a biochemical pathway could be considered to be a nitrogen source. However, the processes of entering the cell and of incorporation must proceed rapidly if they are to contribute significantly to growth. For several amino acids tested in this paper as nitrogen sources for *M. aeruginosa*, they were all taken up rapidly, but not all of them could be assimilated and supported the growth and MC production. Alanine, leucine and arginine did not disrupt the natural metabolism and could be assimilated quickly. They are good nitrogen sources for *M. aeruginosa*. Therefore, they can stimulate the water bloom with high content of MC. Glutamic acid, aspartic acid and lysine seriously disordered the normal physiological metabolism of *M. aeruginosa* and could not promote water bloom and MC production.

These data reported here constitute a basis for investigation into the metabolites that correlate with increased MC production rates and suggest that amino acid assimilation precursors or products may be involved in regulation of MC production. Such information might shed light on the mature of mechanism of up-modulation of MC and the amino acid that play a role in this mechanism. In the present study, individual amino acids were used in medium as nitrogen source. Amino acids supplied together on the growth and MC production will be further studied.

5. Conclusions

This paper studied the effects of six amino acids at their natural concentrations on the growth of the *M. aeruginosa* and the microcystin production in batch culture. *M. aeruginosa* could assimilate alanine, leucine and arginine as sole nitrogen sources for growth and MC-LR production. However, glutamic acid, aspartic acid and lysine could not be assimilated quickly, although they could pass the cell membrane and enter the cell rapidly. Alanine promoted the growth of *M. aeruginosa* and the production of MC-LR than that of arginine and leucine. Leucine could produce more MC than that of arginine although its biomass was less. Arginine was beneficial for the growth of *M. aeruginosa* while not for MC production. In the presence of glutamic acid and aspartic acid, *M. aeruginosa* grew quickly and became yellow and died after 96 h. Therefore, glutamic acid and aspartic acid did not promote the MC-LR production. Lysine did not support the growth and MC-LR production. Our experiment demonstrated that the possible reason of such phenomenon was that different amino acids had different effects on the process of metabolism through the free dissolved amino acids within the cells.

Acknowledgement

This work is supported by the National Basic Research Program of China (Grant No.: 2007CB407301).

References

[1] World Health Organization (WHO), Aquatic (marine and freshwater) biotoxins Environmental Health Criteria, vol. 37, World Health Organization (WHO), Geneva, 1984.
[2] J.M. Rinta-Kanto, A.J.A. Ouellette, G.L. Boyer, M.R. Twiss, T.B. Bridgeman, S.W. Wilhelm, Quantification of Toxic *Microcystis aeruginosa* spp. during the 2003 and 2004 Blooms in Western Lake Erie using Quantitative Real-Time PCR, Environ. Sci. Technol. 39 (2005) 4198–4205.
[3] E. Dittmann, C. Wiegand, Cyanobacterial toxins-occurrence, biosynthesis and impact on human affairs, Mol. Nutr. Food Res. 50 (2006) 7–17.
[4] B.V. Gromov, A.A. Vepritsky, K.A. Mamkaeva, L.N. Voloshko, A survey of toxicity of cyanobacterial blooms in laie ladoga & adjacent water bodies, Hydrobiologia 322 (1996) 149–151.
[5] H. Park, M. Namikoshi, S.M. Brittain, W.W. Carmichael, T. Murphy, Microcystin-LR, a new microcystin isolated from waterbloom in a Canadian Prairie Lake, Toxicon 39 (2001) 855–862.
[6] M.F. Watanabe, S. Oishi, Effects of environmental factors on toxicity of cyanobacterium (*Microcystis aeruginosa*) under culture conditions, Appl. Environ. Microbiol. 42 (1985) 1342–1344.
[7] H.X. Shi, J.H. Qu, A.M. Wang, G.T. Li, P.J. Lei, H.J. Liu, Primary investigation of blue–green algae and their toxins from water blooms in Guanting Reservoir of Beijing, Chem. J. Chin. Univ. 26 (2005) 1653–1655.
[8] S.J. Hoeger, D. Schmid, J.F. Blom, B. Ernst, D.R. Dietrich, Analytical and functional characterization of microcystins [Asp^3]MC-RR and [Asp^3,Dhb^7]MC-RR: consequences for risk assessment, Environ. Sci. Technol. 41 (2007) 2609–2616.
[9] R.J. Wicks, P.G. Thiel, Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir, Environ. Sci. Technol. 24 (1990) 1413–1418.
[10] H.-M. Oh, S.J. Lee, M.-H. Jang, B.-D. Yoon, Microcystin production by *Microcystis aeruginosa* in a phosphorous-limited chemostant, Appl. Environ. Microbiol. 66 (2000) 176–179.
[11] T.G. Downing, C. Meyer, M.M. Gehringger, M. Van de Venter, Microcystin content of *Microcystis aeruginosa* is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate, Environ. Toxicol. 20 (2005) 257–262.
[12] M.V. Ame, D.A. Wunderlin, Effects of iron, ammonium and temperatures on microcystin content by a natural concentrated *Microcystis aeruginosa* population, Water Air Soil Pollut. 168 (2005) 235–248.
[13] L.R. Song, S. Tomoharu, R.H. Li, M. Watanabe, Microcystin production of *Microcystis viridis* (cyanobacteria) under different culture conditions, Phycol. Res. 46 (1998) 19–23.
[14] B.M. Long, G.J. Jones, P.T. Orr, Cellular microcystin content in N-limited *Microcystis aeruginosa* can be predicted from growth rate, Appl. Environ. Microbiol. 67 (2001) 278–283.
[15] K. Kameyama, N. Sugiura, H. Isoda, T. Maekawa, Effects of nitrate and phosphate concentration on production of microcystins by *Microcystis aeruginosa* viridis NIES-102, Aquat. Eco. Health and Manage. 5 (2002) 443–449.
[16] C. Vezie, J. Rapala, J. Vaitomaa, J. Seitsonen, K. Sivonen, Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis aeruginosa* strains and intracellular microcystin concentrations, Microbiol. Ecol. 43 (2002) 443–454.
[17] K.J. Flynn, I. Butler, Nitrogen sources for the growth of marine microalgae: role of dissolved free amino acid, Mar. Ecol. Prog. Ser. 34 (1986) 281–304.
[18] E.I. Butler, S. Knox, M.I. Liddicoat, The relationship between inorganic and organic nutrients in sea water, J. Mar. Biol. Assoc. UK 59 (1979) 239–250.

[19] H. Yan, G. Pan, H. Zou, L.R. Song, M.M. Zhang, Effects of nitrogen forms on the production of cyanobacterial toxin microcystin-LR by an isolated *Microcystis aeruginosa*, J. Environ. Sci. Health 39 (2004) 2003–2993.
[20] D.P. Botes, P.L. Wessels, H. Kruger, M.T.C. Runnegar, S. Stntikarn, R.J. Sith, J.C.J. Barna, D.H. Willianms, Strcctural studies on cyanoginosins-LR, -YR, -YA and -YM, peptide toxins from *Microcystis aeruginosa*, J. Chem. Soc., Perkins Trans. 1 (1985) 2747–2748.
[21] R.L. Charles, M.P. Edward, Free, proteinaceous, and humic-bound amino acids in river water containing high concentrations of aquatic humus, Environ. Sci. Technol. 15 (1981) 224–228.
[22] T. Shoji, Y. Yamamoto, D. Nishikawa, K. Kurihara, H. Ueda, Amino acids in stream water are essential for salmon homing migration, Fish Physiol. Biochem. 28 (2003) 249–251.
[23] P. Xu, Z.Q. Zeng, Uptake of amino acid by toxic *Microcystis aeruginosa* PCC 7820, Acta Hydrobiol. Sin. 18 (1994) 180–182.
[24] R.H. Dai, H.J. Liu, J.H. Qu, X. Zhao, J. Ru, Y.N. Hou, Relationship of energy charge and toxin content of *Microcysytis aeruginosa* in nitrogen-limited or phosphorous-limited cultures, Toxicon 51 (2008) 649–658.
[25] A.S. Ahluwalia, Effects of amino acids and some chemicals on cellular differentiation in *Anabaena oscillarioedes*, Annu. Biol. 1 (1985) 137–144.
[26] B. Palenik, F.M.N. Morel, Comparison of cell-surface L-amino acid oxidases from several marine phytoplankton, Mar. Ecol. Prog. Ser. 59 (1990) 195–201.
[27] B.-H. Cho, E. Komor, The amino acid transport systems of the autotrophically grown green alga *Chlorella*, Biochim. Biophys. Acta 821 (1985) 384–392.
[28] M.F. Turner, Nutrition of some marine microalgae with special reference to vitamin requirement and utilization of nitrogen and carbon sources, J. Mar. Biol. Assoc. UK 59 (1979) 535–552.
[29] J.W. Millbank, Demonstration of transaminase systems in the alga *Chlorella*, Nature (London) 171 (1953) 476–477.
[30] A. Hehmann, K. Kaya, M.M. Watanabe, Selective control of *Microcystis aeruginosa* using an amino acid—a laboratory assay, J. Appl. Phycol. 14 (2002) 85–89.
[31] Y. Yamamoto, T. Kouchiwa, Y. Hodoki, K. Hotta, H. Uchida, K.-I. Harada, Distribution and identification of actinomycetes lysing cyanobacteria in an eutrophic lake, J. Appl. Phycol. 10 (1998) 391–397.