EVALUATION OF BIOLOGICAL DEGRADING CHARACTERISTICS AND MECHANISM OF BACTERIAL COMMUNITY YFMG ON TOXIC MICROCYSTIN-LEUCINE AND ARGININE

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Abstract

The existence of microcystins-leucine (L) and arginine (R) (MC-LR) in freshwater and marine habitats is considered as the most toxic pollutant threatening natural ecosystem and public health, calling for its awareness and treatment. Therefore, it is important to develop approaches capable of reducing and preventing the toxins detrimental consequences. The application of bacteria biological degradation approach is rising owing to its cost effectiveness, safe processes and non-generation of harmful metabolites. Here, a novel MC-LR biological degrading bacterial community named YFMG was isolated and its degrading characteristics and mechanisms against toxic MC-LR were evaluated. Data indicated that MC-LR biological degradation utilizing YFMG was temperature, pH and initial MC-LR concentration dependent, and maximal biological degradation rate occurred at 0.313 µg/mL/hr. MC-LR biological degradation product Adda was identified by HPLC-ESI-MS. High-throughput pyrosequencing analysis revealed that, Proteobacteria and Bacteroidetes were the dominant phyla, and genus Sphingobacterium, Acinetobacter and Stenotrophomonas were enriched in YFMG. Using PCR, YFMG was shown to contain the homologues of mlr gene cluster consisting of mlrABD for MC-LR biological degradation. The biological degrading activities demonstrated by bacterial community YFMG renders it beneficial in biological remediation of the toxins contaminated waterways.

Keywords
MC-LR; Biological degradation; Environmental factors; *mlr* gene cluster; Bacterial community; High-throughput pyrosequencing

Introduction

Microcystins (MCs) are cyclic heptapeptides and are considered as the utmost harmful group of toxins formed during cyanobacterial blooms in drinking and recreational water bodies (Liu et al., 2018; MacKintosh et al., 1990; Massey et al., 2023). All MCs have a collective genetic structure cyclo-(D-Ala-X-D-MeAsp-Z-Adda-D-Glu-Mdha). Adda depicts b-amino acid residue of 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic
acid, Mdha is N-methyldehydroalanine, D-MeAsp is D-erythro-b-methylaspartic acid, while Z and X indicate variable L-amino acids (Bouaicha et al., 2019; Ho et al., 2007; Saito et al., 2003). Miles et al. (2013) reported that, the demethylated or acetylated amino acid residues and variable L-amino acids led to the identification of numerous variants of MC. Of the approximately 279 MC variants reported, MC-leucine (L) and arginine (R) (MC-LR) is the utmost abundant, very lethal and extensively disseminated, and it has become the focus of global research (Bouaicha et al., 2019; Massey et al., 2023; Yang et al., 2018b).
Exposure to MC-LR is established to affect the various mammalian organs including liver, lung, heart, brain, kidney, intestines and reproductive system, inducing diverse diseases as well as death (Ho et al., 2007; Massey et al., 2023; Saito et al., 2003). MacKintosh et al. (1990) demonstrated that, the toxin is capable of impacting the aforementioned health risks via the inhibition of serine/threonine protein phosphatases 1 and 2A.
To circumvent MC-LR health risks, the World Health Organization (WHO) recommended a provisional 1 µg/L toxin guidelines for drinking water quality (WHO, 2004) and maximum 20,000 cyanobacterial cells/mL or 10 µg/L of chlorophyll-a where about 2 – 4 µg/L of the toxin is anticipated) guidelines for safe recreational water environment (WHO, 2003). Consequently, it is essential to develop approaches capable to reduce and prevent the hazards posed by this toxin. The physical and chemical water treatment technologies over the past decades provided some solutions for removing dissolved MC-LR from contaminated water, nonetheless, the treatment technologies were associated with low removal efficacy, high operational costs and harmful by-products (Lawton and Robertson, 1999; Massey and Yang, 2020; Svrcek and Smith, 2004). The utilization of bacteria biological degradation approach is rising due to its safe procedures, cost effectiveness and non-generation of harmful metabolites (Bouaicha et al., 2019; Ho et al., 2007; Massey and Yang, 2020; Saito et al., 2003). Till present, few scholars have indicated that bacterial community to a certain degree could be more suitable in biologically degrading MC compared to single bacteria strain (Massey and Yang, 2020; Mou et al., 2013; Tsao et al., 2017). For this reason, acquiring bacterial community for MC-LR biological degradation has become vital to explore the knowledge on bacterial community biological degradation of the toxin.
In our earlier study, a toxigenic *Microcystis* sp. YFM1 was obtained from eutrophic water located in Hunan Province,

China and it was found to produce different variants of MC including MC-LR (Liu et al., 2018). In this study, bacterial community termed YFMG was also successfully obtained from the eutrophic water. The biological degrading characteristics of YFMG were assessed under various environmental factors (temperature, pH and initial MC-LR concentration). Based on the polymerase chain reaction (PCR) technology, the toxins biological degrading genes in YFMG that revealed the biological degradation potential were investigated. Further, high-throughput pyrosequencing was used to analyze the structure of YFMG.

Materials and Methods

Materials and reagents

Standard MC-LR with purity $\geq$ 95% was purchased from Alexis Corporation (Lausen, Switzerland) and stored at -20°C. Trifluoroacetic acid and methanol utilized for high performance liquid chromatography (HPLC) were bought from Dikma Technology Incorporation (Foothill Ranch, California, USA). Mineral salt medium (MSM, pH 7) used for bacterial culture, acquisition and MC-LR biological degradation containing (g/L) $MgSO_4.7H_2O$ 1.0; KH_2PO_4 0.5; K_2HPO_4 4.0; NaCl 1.0; $CaCl_2$ 0.02; $FeSO_4$ 0.005; $MnCl_2.4H_2O$ 0.005; $ZnCl_2$ 0.005; $CuCl_2$ 0.0005 was obtained from Wanqing Chemical Co., Ltd. (Nanjing, China). Phosphate buffer saline (PBS, pH7) medium containing NaCl, $Na_2HPO_4.12H_2O$, Na $H_2PO_4.2H_2O$, 10 mM was attained from Wanqing Chemical Co., Ltd. (Nanjing, China). Nutrient agar (NA) contained 0.3% beef extract, 0.5% peptone, 0.5% sodium chloride and 1.5% agar. Nutrient broth (NB, pH 7) medium used for bacteria growth contained 1 g peptone, 0.5 g sodium chloride and 0.5 g beef extract (Yang et al., 2018a,b). The experiment was conducted at the Hunan Provincial Key Laboratory of Clinical Epidemiology, Xiangya School of Public Health, Central South University, Changsha, China.

Bacterial community isolation

From a eutrophic water located in Changsha City of Hunan Province, China, 5 g of wet weight sludge sample was obtained during the bloom of *Microcystis* (28° 101.31" N and 112° 560.07" E) determined by geographical positioning system. Sterile water (45 mL) was added to 5 mL of each sample and for 30 min, it was shaken at a constant condition (120 rpm and 30°C). Allowing it to stand still for 15 min, the supernatant (5 mL) was inoculated into 45 mL MSM which contained MC-LR. 5 mL resulting solution was inoculated into 45 mL MSM with the toxin to form a new subculture after 192 h. Repeating the procedure four times under similar conditions, the final subculture was inoculated onto MSM agar plates which contained 10 μg/mL standard MC-LR, and it was purified by plate-streaking technology on the MSM agar plate. A novel bacterial community exhibiting high toxin biological degrading characteristics was aquired and termed as YFMG (Yang et al., 2018a,b).

Biological degrading characteristics of bacterial community on MC-LR

Assessing the biological degrading characteristics of YFMG on MC-LR, the bacterial community was cultured for 72 h in NB medium in a 100 mL distilled water at 30°C as earlier demostrated (Yang et al., 2018a). The bacterial community after obtaining OD600 value of 0.6 via SpectraMaxPlus 384 (Molecular Device, Silicon Valley, CA, USA), centrifugation (5,000 x g, 15 min, 4°C) was performed to harvest the bacterial cells. Disposing the supernatant, washing procedure was repeated thrice using 10 mM PBS, and centrifuged. The acquired cells were re-suspended into MSM which contained MC-LR and cultivated under varying incubation conditions (environmental factors), determined at temperature 20°C, 30°C or 40°C, pH 5, 7 or 9, and initial MC-LR concentration of 1 μg/mL, 3 μg/mL or 5 μg/mL. Each procedure was conducted thrice, and samples devoid of bacteria were used as control. Throughout the procedure, 50 μl samples were withdrawn every 4 h for 16 h and centrifuged (12,000 x g, 15 min, 4°C). HPLC was utilized in monitoring the toxin concentrations in the samples.

Determination of MC-LR and biological degrading product

MC-LR and biological degrading product were investigated by Agilent 1100 HPLC machine with a Zorbax Extend C18 column (4.6 x 150 mm, 5 μm, Agilent, Palo Alto, CA, USA) and a variable wavelength detector set at 238 nm. The mobile phase was a combination of 0.1% trifluoroacetic acid aqueous solution and methanol (45:55, v/v). The flow rate was 1 mL/min, injection volume was 10 μl and column temperature was set at 40°C. The qualitative analysis of the toxin in the sample was carried out according to the retention time (Rt) in the chromatogram and the maximum absorption wavelength (λmax) of the spectrum. The peak area of the chromatogram was quantitatively analyzed by external standard method.
The biological degrading product of the toxin was identified by HPLC coupled with an ultra-high resolution LTQ Orbitrap Velos Pro ETD mass spectrometry (Thermo Scientific, Dreieich, Hesse, Germany) equipped with electrospray ionization interface (LTQ Orbitrap Velos Pro ETD, Thermo Fisher, Waltham, MA, USA) (HPLC-ESI-MS). While the auxiliary gas was set at a flow rate of 30 psi, the sheath gases was set at a flow rate of 5 psi. The dry gas temperature and nebulizer pressure were set at 350°C and 45 psi respectively. Spectra were documented in positive modes at a spray voltage of 3.5 kV (Yang et al., 2018a,b).

Detection of mlr genes in bacterial community

Amplifying for *mlrA*, *mlrB*, *mlrC* and *mlrD* genes in the bacterial community employed oligonucleotide primer sequences illustrated in Table 1. The PCR reaction system was 50 μL, which included 25 ng of template DNA, 1 x PCR buffer (20 mM Tris-HCL, pH 8.4; 50 mM KCL), 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 4 μM of each primer, and 1.25 units of Taq DNA polymerase. The PCR condition comprised the

Table 1. Oligonucleotide primer sequences for the genes *mlrA*, *mlrB*, *mlrC* and *mlrD*

Gene	Primers	Sequences (5'-3')	Reference
mlrA	MF	GACCCGATGTTCAAGATACT	Saito et al. (2003)
	MR	CTCCTCCCACAAATCAGGAC	
mlrB	mlrBf1	CGACGATGAGATACTGTCC	Ho et al. (2007)
	mlrBr1	CGTGCGGACTACTGTTGG	
mlrC	mlrCf1	TCCCCGAAACCGATTCTCCA	Ho et al. (2007)
	mlrCr1	CCGGCTCACTGATCCAAGGCT	
mlrD	mlrDf1	GCTGGCTGCGACGGAAATG	Ho et al. (2007)
	mlrDr1	ACAGTGTTGCCGAGCTGCTCA	

initial denaturation at 94°C for 5 min; 94°C for 30 sec, annealing for 30 sec at 56°C, 30 cycles of extension for 1 min at 72°C; and a final elongation for 7 min at 72°C and held at 4°C. Agarose gel electrophoresis was used to extract the PCR products.

Analysis of bacterial community

Bacterial genomic DNA extraction kit (Biocolor BioScience and Technology Company, China) was employed in extracting genomic DNA of the bacterial community. The DNA extracts were stored at -20°C. The 16S rDNA fragment was amplified by PCR, via the universal bacteria primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTAC HVGGGTWTCTAAT-3') (Su et al., 2016). The PCR reaction was performed as follows: 95°C for 4 min; 30 cycles at 95°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and finally 72°C for 6 min. PCR products were submitted to Novogene (Beijing, China) for pyrosequencing analysis.

Results and Discussion

Results

Bacterial community isolation

An efficient bacterial community, exhibiting the toxins biological degrading ability was isolated from the eutrophic water, and denoted as YFMG. The bacterial community was used for MC-LR biological degradation.

Biological degrading characteristics of YFMG on MC-LR

Figure 1 illustrates the biological degradation characteristics of YFMG on MC-LR. Data in Figure 1a indicated that at pH 7, YFMG biologically degraded 3 μg/mL initial MC-LR at a rate of 0.124 μg/mL/h, 0.188 μg/mL/h and 0.153 μg/mL/h, at temperature 20°C, 30°C and 40°C, respectively, in 16 h. Data in Figure 1b uncovered that at temperature 30°C, YFMG biologically degraded 3 μg/mL initial MC-LR at a rate of 0.078 μg/mL/h, 0.188 μg/mL/h and 0.188 μg/mL/h, at pH 5, 7 and 9, respectively, in 16 h. Data in Figure 1c showed that at pH 7 and temperature 30°C, YFMG biologically degraded 1 μg/mL initial MC-LR, 3 μg/mL initial MC-LR and 5 μg/mL initial MC-LR at a rate of 0.125 μg/mL/h, 0.188 μg/mL/h and 0.313 μg/mL/h, respectively, in 16 h. The toxins concentrations in samples measured by HPLC is demonstrated in Supplementary Table 1. From the findings, 0.313 μg/mL/h was revealed as the maximum biological degrading rate, which was realized at 5 μg/mL initial MC-LR, 30°C and pH 7. In the control samples devoid of YFMG, biological degradation of MC-LR was lacking.

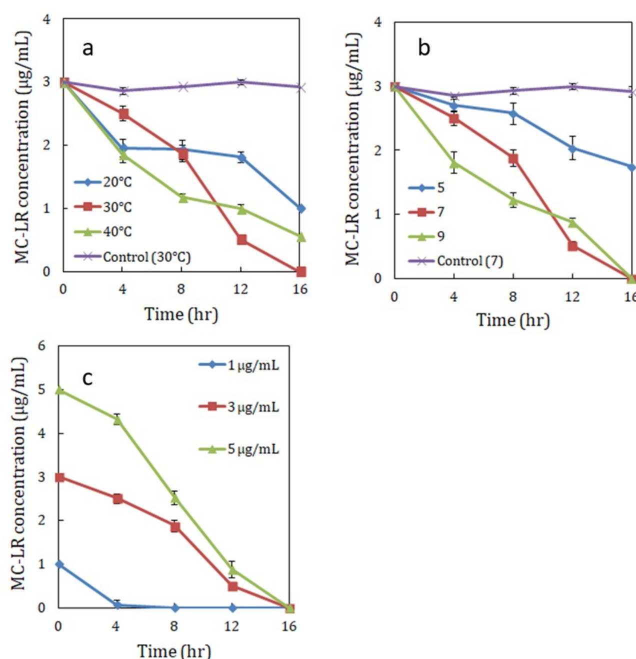

Figure 1. Biological degradation characteristics of YFMG on MC-LR. The error bars reveal the standard deviation of three replicates.

Determination of MC-LR and its biological degradation product

Biological degradation of MC-LR by YFMG was evaluated in culture under the optimal conditions (5 μg/mL initial MC-LR, 30°C and pH 7). Figure 2 shows the results of HPLC chromatograms of MC-LR and its biological degradation product. Results in Figure 2a demonstrated that the toxin's Rt was 15.8 min. After incubation, the peak area of the toxin extensively

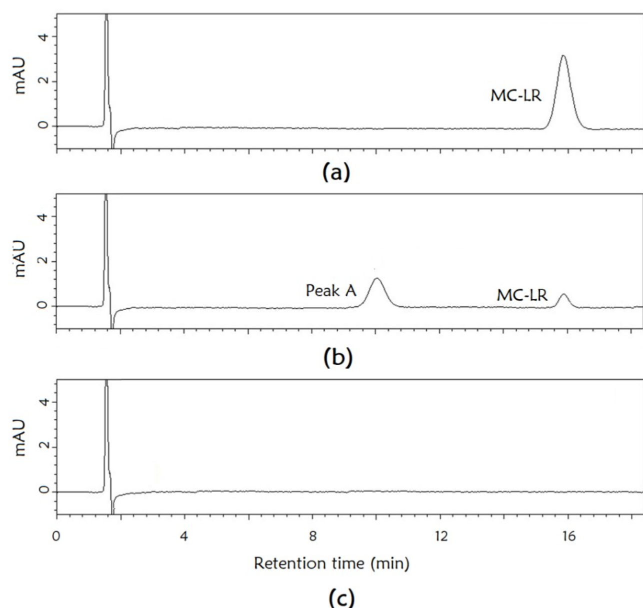

Figure 2. HPLC chromatograms obtained during the biological degradation of MC-LR incubated with YFMG at time 0 h (a), 10 h (b) and 16 h (c)

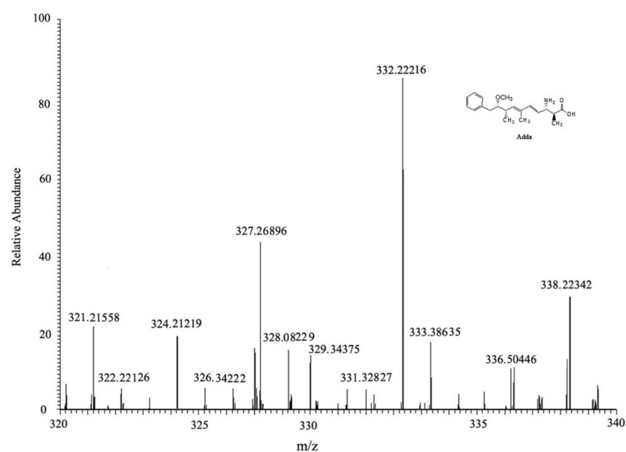

Figure 3. HPLC-ESI-MS spectrum and putative structure of the biologically degraded product of MC-LR

declined and a main intermediate product (peak A) was noticeable at 10 h (Figure 2b). Results in Figure 2c depicted the disappearance of all peaks, signifying complete biological degradation of the toxin and its product in 16 h. HPLC-ESI-MS was used to further classify the observed product (peak A) and exhibited accompanying ion at m/z 332.22216 (Figure 3).

Detection of *mlr* genes in YFMG

mlr gene cluster consisting of *mlrA*, *mlrB* and *mlrD* were detected in YFMG using PCR (Figure 4). The clone gene sequences were registered and deposited in GenBank with accession numbers MK213111 (*mlrA*), MK213112 (*mlrB*) and MK213113 (*mlrD*) to attain more definitive information on the *mlr* gene cluster.

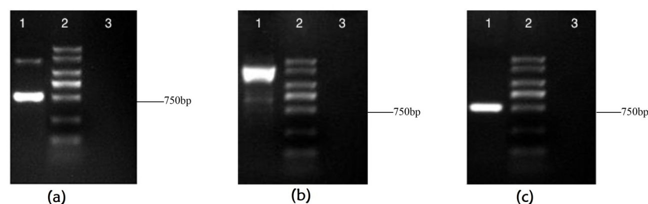

Figure 4. The presence of functional genes involve in MC-LR biological degradation (a) *mlrA*, (b) *mlrB*, (c) *mlrD* where 1 is YFMG; 2 is marker; 3 is negative control

Performing nucleotide sequences similarity via the National Center for Biotechnology Information database (`http://www.ncbi.nlm.nih.gov/BLAST`), the MEGA Software (fourth version) developed by Tamura et al. (2007) was applied to generate a phylogenetic tree utilizing the neighbor-joining method (Figure 5).

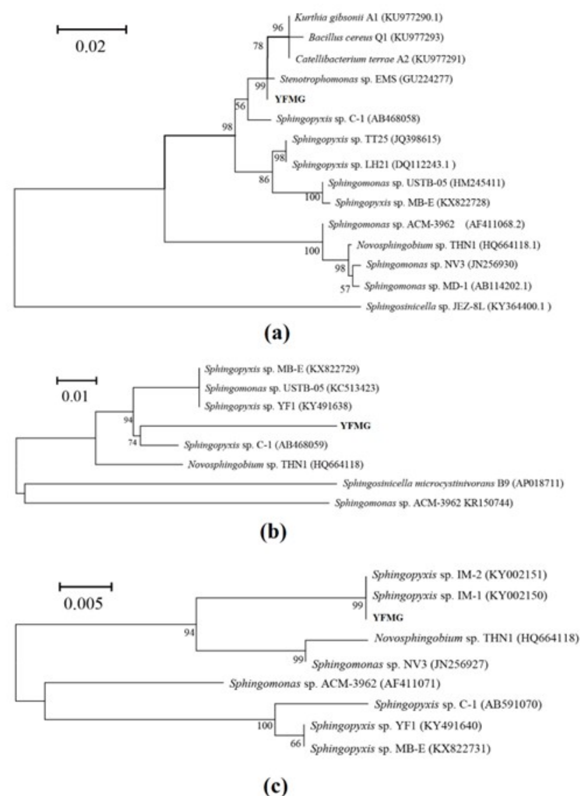

Figure 5. A phylogenetic tree of the cloned mlr gene sequence of YFMG using neighbor-joining technique (a)*mlrA*, (b)*mlrB* and (c)*mlrD*. The numbers at the nodes are the levels of bootstrap support (%) based on the neighbor-joining analysis of 1,000 resample data sets. The scale bar represents 0.02, 0.01 and 0.005 nucleotide substitutions per position respectfully.

Data discovered that, the functional *mlrA* from YFMG was 99.41% similar to *mlrA* gene of *Stenotrophomonas* sp. EMS (GU224277) and 98% similar to *mlrA* gene of *Sphingopyxis* sp. C-1 (AB468058). The *mlrB* was 94% similar to *mlrB* gene

of *Sphingopyxis* sp. C-1 (AB468059) and 93% similar to *mlrB* gene of *Sphingomonas* sp. USTB-05 (KC513423). The *mlrD* was 99.42% similar to *mlrD* gene of *Sphingopyxis* sp. IM-1 (KY002150) and 94% similar to *mlrD* gene of *Sphingopyxis* sp. C-1 (AB591070). Bootstrapping procedure using 1000 random samples was applied to assess the phylogenetic tree.

Analysis of YFMG

The sequence analysis procedure carried out under the optimal conditions (5 μg/mL initial MC-LR, 30oC and pH 7 for 16 h) revealed that Total Tag, Taxon Tag, Unique Tag and OTU in YFMG were 79393, 78519, 874 and 343 respectively. At phylum level, YFMG was found to be composed of *Proteobacteria*, *Bacteroidetes* and others (*Firmicutes*, *Actinobacteria*, *Spirochaetes*, *Deinococcus-Thermus*, *Fusobacteria*, *Acidobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*) (Figure 6). *Acinetobacter*, *Sphingobacterium*, *Aeromonas*, *Stenotrophomonas*, *Alcaligenes*, *Pseudomonas*, *Methylobacillus*, *Phyllobacterium*, *Flavobacterium*, and *Escherichia-Shigella* were among the genus indentified in YFMG (Figure 7).

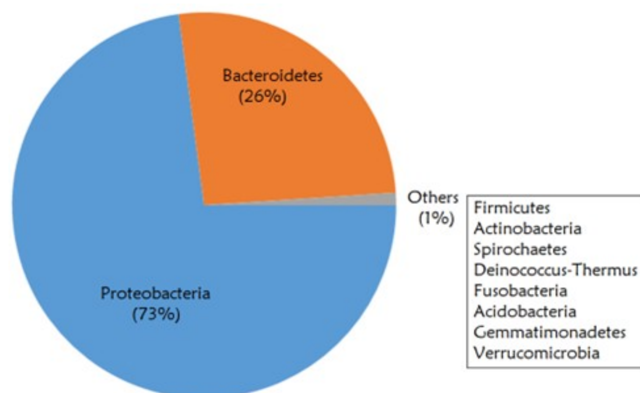

Figure 6. Bacterial community structure at phylum level

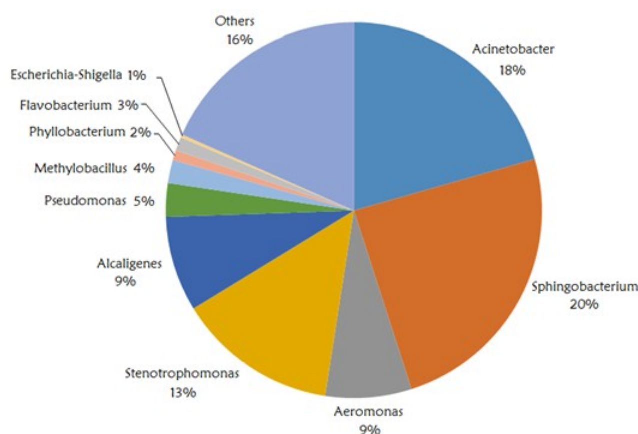

Figure 7. Bacterial community structure at genus level

Discussion

The existence of MC-LR in water has been considered as the most toxic pollutant threatening natural ecosystem and public health, calling for the awareness of its treatment. Some researchers have isolated bacterial communities and demonstrated their biological degrading characteristics (Massey and Yang, 2020; Mou et al., 2013; Ramani et al., 2012).

From a eutrophic water situated in Changsha City, an efficient bacterial community designated as YFMG was obtained for MC-LR biological degradation. YFMG which displayed a maximal MC-LR biological degradation rate at 7.512 μg/mL/day was discovered to be higher than the bacterial community reported by Cousins et al. (1996) (1.4 x 10^3 μg/mL/day), Ramani et al. (2012) (0.18 μg/mL/day) and Tsao et al. (2017) (0.876 μg/mL/day). However, the biological degradation rate of YFMG was much lower than that of Yang et al. (2018a,b) (12 μg/mL/day) and Manheim et al. (2018) (50 μg/mL/day). The difference in the biological degradation rates of the toxin may be due to the alteration in bacterial strains contained in the bacterial community, bacterial community concentration, MC-LR type and concentration applied for the experiments as well as other chemicals and physical conditions used in the laboratory.

Studies have established that, diverse environmental factors including initial MC-LR concentrations, temperatures and pH take part in an essential responsibility regarding the toxin's biological degradation procedure. Using diverse bacterial communities, Cousins et al. (1996) indicated 10 μg/mL initial MC-LR at 21°C, pH 7, Ramani et al. (2012) 0.25 μg/mL initial MC-LR at 26°C, pH 7, Mou et al. (2013) found 15 μg/mL initial MC-LR at 22°C, pH 7, Tsao et al. (2017) demonstrated 5 μg/mL initial MC-LR at 30°C, pH 7.6, Yang et al. (2018a,b) unveiled 5 μg/mL initial MC-LR at 30°C, pH 7 and Manheim et al. (2018) uncovered 200 μg/mL initial MC-LR at 24°C, pH 7 as the maximum toxin's biological degrading conditions. In this investigation, the aforementioned environmental factors were indicated to significantly impact the biological degradation process, and 5 μg/mL initial MC-LR at 30°C, pH 7 was determined as the maximum toxins biological degradation conditions. Apparently the pH and temperature conditions were noted to be more suitable to work for YFMG favouring the total biological degradation of the toxin. However, the optimum biological degradation conditions obtained cannot be generalized as the toxin concentration can be higher, and calls for additional investigation.

Adda has established its necessity for the characteristic biological activities of MC (Massey and Yang, 2020; Tsao et al., 2017; Yang et al., 2018a,b). In the present study, a main biological degradation product (Adda) appeared when YFMG biologically degraded the toxin and exhibited accompanying ion at m/z 332.22216. This was identical to Adda that was the intermediate toxin biological degradation product of the bacterial community reported by Tsao et al. (2017) and Yang et al. (2018a,b). MC-LR and Adda residue disappearing after 16 h implied that, YFMG has high toxin biological degrading capability. Nevertheless, the biological degradation product (Adda) needs to be further studied and clarified.

The *mlr* gene cluster is recognized as the toxins biological de-

grading gene, made up of *mlrA*, *mlrB*, *mlrC* and *mlrD*. These mlr gene clusters encodes the most important enzyme required for MC-LR, linearized MC-LR, and tetrapeptide biological degradation (Massey and Yang, 2020; Tsao et al., 2017; Yang et al., 2018a,b). Previous acquired bacterial communities were correlated to mlr gene levels for the toxins biological degradation (Massey and Yang, 2020; Tsao et al., 2017; Yang et al., 2018a,b). Data from this study also indicates that, YFMG possesses *mlrA*, *mlrB* and *mlrD* genes. This suggests that, the MC-LR biological degrading mechanism of YFMG is similar to earlier isolates (Massey and Yang, 2020; Mou et al., 2013; Tsao et al., 2017; Yang et al., 2018a,b).

Prior scholars (Massey and Yang, 2020; Yang et al., 2018a,b) found that, the MlrA enzyme (identified as putative metalloprotease) encoded by *mlrA* gene in the biological degradation pathway is the first enzyme to hydrolyze and open the cyclic MC-LR into a linear intermediate. The presence of this gene in YFMG confirms the hypothesis that *mlrA* gene is probably highly relevant to the toxins biological degradation. The MlrB enzyme (belonging to the serine protease family) encoded by *mlrB* gene is believed to catalyze hydrolysis of the resultant linear structure of the toxin. In this study, linearized MC-LR was not identified possibly due to its low concentration or rapid biological degradation, making it difficult for HPLC-MS to detect. In addition, the MlrD enzyme (identified as a putative oligopeptide transporter) encoded by *mlrD* gene is considered to be responsible for the uptake of the toxin into the bacterial cell. Since its function is still speculative, further studies are required to elucidate the uptake of MC-LR into the bacterial cell. It is of interest that, the MlrC enzyme encoded by *mlrC* gene thought to catalyze further hydrolysis of the toxin into smaller peptides and then into amino acids was not detected in YFMG. As evident, MC-LR and its biological degradation product (Adda) gradually decreased with incubation time and eventually no detectable amount of MC-LR or Adda was observed. This implies, how the toxin was further biologically degraded into some undetectable smaller peptide fragments and amino acid is worth investigating. It is therefore assumed that the primer sequences used might not be good enough in detecting the *mlrC* gene in YFMG. Although how MC-LR was further biologically degraded still remain unknown, feasible reasons may include (1) *mlrA* and *mlrB* genes may have possessed the function of *mlrC* gene to biologically degrade tetrapeptide into Adda and (2) an unknown mechanism may have involved in the further biological degradation of the resultant linear structure of MC-LR aside the *mlrB* gene. The tetrapeptide was also not revealed probably owing to its low concentration or speedy degradation making it unfavorable for HPLC-MS to recognize. In view of this, the biological degradation pathway of the toxin by YFMG was proposed Figure 8.

The composition of YFMG was subsequently examined, as the toxins biological degradation efficiency is influenced by the functions and behaviors of bacteria (Su et al., 2016). Utilizing high-throughput pyrosequencing technology, a total of 11 phyla dominated by *Proteobacteria* and *Bacteroidetes* were found (Figure 6). *Proteobacteria* and *Bacteroidetes* have been identified by a number of researchers as the major phyla in bacterial communities (Massey and Yang, 2020; Tsao et al., 2017; Yang et al., 2018a,b). It is therefore not surprising these two phyla were uncovered as the most dominant in YFMG. The high composition of these phyla suggest their distinctive enrichment, playing an important role in the biological degradation process.

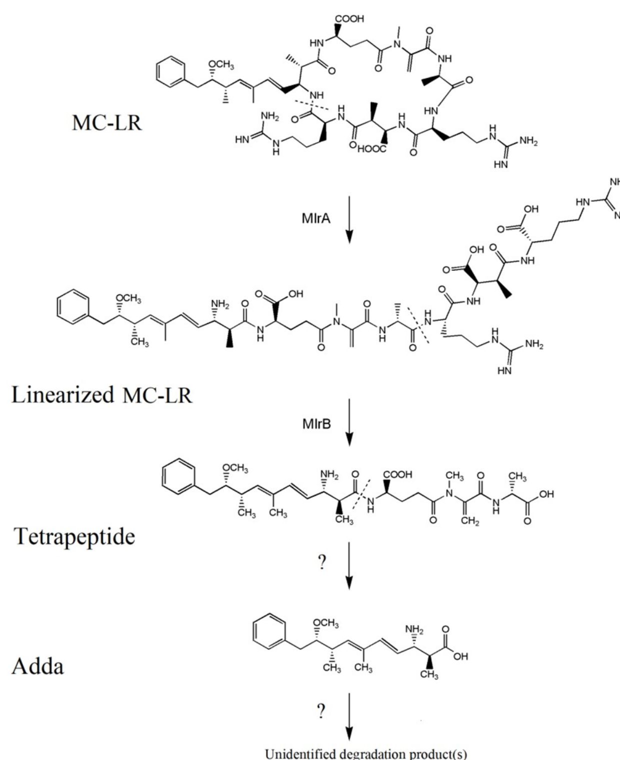

Figure 8. Proposed biological degradation pathway of MC-LR showing the formation of identified intermediate product (Adda) by YFMG. The short dashes represent sites of peptide hydrolysis

Further at genus level, YFMG was highly enriched in *Sphingobacterium*, *Acinetobacter* and *Stenotrophomonas*. The genus *Alcaligenes* and *Aeromonas* were equally distributed, while *Escherichia-Shigella* was the least detected (Figure 7). Reports shows that, the genus identified in YFMG have the ability of biologically degrading various variants of MCs (Massey and Yang, 2020; Mou et al., 2013; Ramani et al., 2012; Yang et al., 2018a,b). This further suggests that, the genus had a significant influence on the toxin during the biological degradation process. Also bacteria concentration variations, and interactions between bacteria and other microorganisms could have contributed to the effectiveness of YFMG (Su et al., 2016). Consequently, any change in the structure of YFMG is capable of affecting the toxin's biological degradation efficiency. However, further works need to investigate the biological degrading characteristics and mechanisms of the various genera detected YFMG.

Limitations of the study

The current study did not (1) adopt any other method of toxin purification aside the biological method, (2) detect *mlrC* gene in YFMG, (3) identify tetrapeptide and (4) investigate the biological degrading characteristics and mechanisms of the genera discovered in YFMG.

Conclusion

MC-LR biological degrading bacterial community identified as YFMG was aquired from eutrophic water located in Hunan Province, China. High-throughput *pyrosequencing* showed *Proteobacteria* and Bacteroidetes as the major phyla and at genus level *Sphingobacterium*, *Acinetobacter* and *Stenotrophomonas* were dominant in YFMG. The highest MC-LR biological degradation rate of 0.313 μg/mL/h using YFMG was extensively affected by the varying environmental factors. The main intermediate biological degradation product Adda was also biologically degraded by YFMG. *mlrA*, *mlrB* and *mlrD* genes that encode the most important enzyme and observed to play a vital role in MC-LR biological degradation were observed in YFMG. To the best of our knowledge this is the first study on MC-LR biological degradation utilizing bacterial community from eutrophic water in Hunan Province.
The data demonstrated thorough MC-LR biological degradation using bacterial community YFMG contributing to the biological treatment of water bodies contaminated by the toxin. However, the absence of *mlrC* gene in YFMG has paved way for additional investigations on the biological degradation mechanism. Studies are required to elucidate the down-stream route biological degradation of the resultant linear MC-LR structure into Adda.

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Conflict of interest

The authors declare no conflict of interest

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