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Original Article

Systematic identification of seven ribosomal protein genes in bighead carp and their expression in response to microcystin-LR

Yan Cai[1], Chao Zhang[1], Le Hao[3,4], Jun Chen[2], Ping Xie[2] and Zhidong Chen[1]

[1]School of Petrolchemical Engneering, Changzhou University, Changzhou 213164, China
[2]Donghu Experimental Station of Lake Ecosystems, State Key Laboratory for Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[3]Guangdong Public Laboratory of Veterinary Public Health, Institute of Animal Health, Guangdong Academy of Agricultural Sciences, Guangzhou 510642, China
[4]South China Agricultural University, Guangzhou 510642, China

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ABSTRACT — Microcystin-LR (MCLR) is one of the most toxic cyanotoxins produced in algal blooms. The toxic effects of MCLR on the expression of some organelles genes (mitochondrion, endoplasmic reticulum, and cytoskeleton etc) have been widely investigated, but little is known how it impacts on the expression of ribosomal genes. In this study we identified seven ribosomal protein genes RPS6, RPS12, RPS24, RPS27a, RPL12, RPL27 and RPL29 in bighead carp (*Aristichthys nobilis*), whose expression was regulated by MCLR. The amino acid sequences of those 7 genes shared more than 90% identity with corresponding sequences from zebrafish, and were well conserved throughout evolution. The 3D structure prediction showed that the structures of these ribosomal proteins were conserved, but had species specificity. Q-PCR analysis revealed that expression of seven genes changed dramatically at 3 hr, then went back to a moderate change- level at 24 hr in almost all tested tissues (liver, kidney, intestine, heart, spleen and gill) post MCLR injection, but in brain expression of the seven genes stayed same as the normal level. This study will help us to know not only about the evolution and functions of ribosomal proteins in anti-MCLR response in bighead carp, but also about the MCLR toxicity and its impact on aquaculture and human health.

Key words: Ribosomal protein, Microcystin-LR, Gene identification, Bighead carp

INTRODUCTION

Toxin-producing algal bloom is one of the major public health and ecological concerns. High concentration cyanotoxins are often produced in surface waters, threatening food safety and human health. Among these toxins, microcystins (MCs) are a diverse group of cyclic heptapeptide toxins, and their main target organ is liver. MCLR is the most toxic variant of microcystins, and the structure is showed in Fig. 1 (Yu *et al.*, 2015). It inhibits the activity of the serine/threonine protein phosphatase PP1 and PP2A, interrupts signaling transduction, induces oxidative stress, and disrupts Na^+/K^+ ATPase pumps (Alexandre and Vitor, 2010). Many published papers have described that microcystins could cause dysfunction in mitochondrion, endoplasmic reticulum, and cytoskeleton etc (Rogers *et al.*, 2011; Zhang *et al.*, 2013; Zhou *et al.*, 2015). The expression of many proteins of these organelles has been affected due to MCLR exposure (Chen *et al.*, 2013; Rymuszka, 2013). The structure and function of ribosome are tightly connected with other cellular organelles such as mitochondrion, endoplasmic reticulum, and cytoskeleton (Zhou *et al.*, 2015; Zhao *et al.*, 2015), but the impact of ribosomal structure and function by microcystins is still unknown, especially the change of ribosomal protein expression.

Ribosomes are one of the vital organelles in cells. They translate the genetic information from mRNA into protein, and regulate many other processes of cellular metabolism. Ribosomes in eukaryotes are made up of 4

Correspondence: Zhidong Chen (E-mail: chen@cczu.edu.cn)

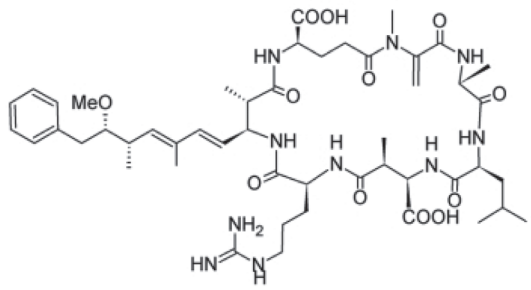

Fig. 1. The chemical structure of microcystin-LR (Yu *et al*., 2015).

rRNAs and 79 proteins. The protein components, also known as ribosomal proteins (RPs), have different functions in every species. One of their primary functions is playing a critical role in ribosome synthesis and function, and acting as a scaffold that enhances the catalytic ability of rRNA to synthesize protein. Several RPs perform important extra-ribosomal functions, being involved in DNA repair, transcriptional regulation and apoptosis (Chang *et al.*, 2015). Misexpression of human cytoplasmic ribosomal protein (CRP) and mitochondrial ribosomal protein (MRP) genes results in many human syndromes and diseases, including Diamond-Blackfan anaemia, Turner syndrome, hearing loss and cancer (Narla and Ebert, 2010). Many RPs are conserved from bacteria to humans, and their peptide and nucleotide sequences are useful for studying phylogenetic relationships (Lam and Trinkle-Mulcahy, 2015).

The first identified cytoplasmic RPs were isolated from the rat cytoribosome. Individual RPs were separated by two-dimensional gel electrophoresis at first and named from their origin in the Small or Large subunit and their relative electrophoretic migration positions. In 2004 seventy-nine distinct mammalian CRPs were identified. Their amino acid sequences and biochemical properties have been described (Nakao *et al.*, 2004). At present, many CRPs and MRPs from different species have been identified (http://ribosome.miyazaki-med.ac.jp/). However, to date, no data have been reported in term of RPs in bighead carp.

Bighead carp (*Aristichthys nobilis*) not only is a commercially important fish in China, but also consumes great quantities of toxic blue-green algae, which may contain high concentrations of microcystins. As a freshwater phytoplanktivorous fish, compared to mammals, bighead carp is more resistant to the toxic effects of MCLR (Li *et al.*, 2014). Today bighead carp has been introduced into more than 20 countries, primarily for the purposes of aquaculture and algal control. Now attention is being paid to bighead carps for aquaculture production and as a model species in research on toxicology, ecology, physiology, evolutionary genetics, and nutrition (Xie, 2003; Sun *et al.*, 2016).

In our previous study, we identified some genes whose expression was changed in bighead carp liver due to MCLR exposure. In all these genes, seven genes were RP genes based on NCBI genbank database (unpublished data). To better understand MCLR-induced toxicity on RP genes expression, as well as the evolution of RP genes, we screened a hepatic cDNA library through suppression subtractive hybridization (SSH), which was constructed with the mixed livers of bighead carps at different time points post 200 μg per kg bw (body weight) MCLR injection (unpublished data), and isolated seven cDNA fragments, which indicated high homology to zebrafish ribosomal protein cDNA. Expression of three RPs, RPS12, RPS24 and RPL12, was down-regulated, while expression of the four other proteins, RPS6, RPS27, RPS27a and RPL29, was up-regulated in SSH. Using rapid amplification of cDNA ends (RACE) method, we obtained the full-length cDNA of the seven proteins. And the molecular characteristics of the RPs were further analyzed. Their mRNA expression profiles in vital organs at 3 hr and 24 hr post 200 μg per kg bw MCLR injection were also investigated.

MATERIALS AND METHODS

Fish preparation

Healthy bighead carps (body weight 899 ± 251 g, no medical history) were purchased from a local cauf with no MCLR contamination in Wuhan, China, and acclimated for 2 weeks with 6 bighead carps (half male and half female) in each group. The experiment was conducted in the following conditions: water temperature 25 ± 1°C, light cycle 12L:12D, dissolved oxygen 6.8 ± 0.7 mg/L, pH 7.4 ± 0.9. In the acclimation period the dry food given to the bighead carps was 2% of body weight, and no food was supplied 2 days before and during the course of the experiment.

Toxin

MCLR was isolated from surface cyanobacterial blooms (mainly *Microcystis aeruginosa*) collected from Lake Dianchi in China, then purified and quantified using an improved Ramanan method (Hao *et al.*, 2008). MCLR was dissolved in water, and the purity was above 97%.

Sampling

According to the method of Li *et al*. (2005), one group were injected intraperitoneally (i.p.) along the ventral midline using syringes with 200 μg MCLR / kg bw. No mortality was found during the experimental period. The 6 carps in the other group were injected i.p. with the same volume of distilled water as control. In the experiment, sampling time points were at 3 hr and 24 hr post MCLR injection. Three replicates of tissue were from different fish at each sampling time point. Approximately 100 mg of liver, kidney, intestine, brain, heart, spleen and gill were excised, freed of attached tissue, and respectively stored in 1 mL Trizol (Invitrogen, NewYork, CA, USA) at -70°C. All of the experiments on bighead carp were performed with the approval of the animal ethics committee at the Institute of Hydrobiology, Chinese Academy of Sciences (Study ID# Y11309-1-201).

RNA extraction and Q-PCR

Isolation, purification and quantification of total RNA, first-strand cDNA synthesis, and semi-quantitative real time PCR (Q-PCR) were performed using our previously described protocols (Cai *et al.*, 2012). On the basis of the results of our previous studies, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression level was not significantly different between control and treated groups following the treatment with MCLR, and it was chosen as an internal control to normalize the data. The primers used in Q-PCR were listed in Table 1. The specification of each pair of primers was confirmed by randomly sequencing six clones, and further confirmed by melting curve analysis using Q-PCR. The amplification efficiency of each pair of primers was tested by normalizing to corresponding plasmids and only primers with the similar amplification efficiency were used in this experiment. The data obtained from the Q-PCR analysis were subjected to one-way analysis of variance (one-way ANOVA) using SPSS 13.0 software. Differences were considered significant if $P \leq 0.05$.

cDNA fragments of ribosomal protein genes and RACE

Seven RPs cDNA fragments were initially isolated from the hepatic cDNA library of bighead carp through SSH. Total hepatic RNA was used as template to amplify the 5' and 3' ends of cDNAs of these genes. All primers used in the RACE method were listed in the Supplementary Table 1. RACE was performed using SMART RACE cDNA Amplification kit (Clontech, NewYork, CA, USA) according to the manufacturer's instruction. Gene specific primers were designed based on the sequences of cDNA fragments, with the help of Primer 5.0 software. Briefly, the primers, UPM (long and short), 5'CDS and genes specific downstream primers were applied respectively for 5' RACE. For 3' RACE, the cDNA template was first transcribed by AMV Reverse Transcriptase (Takara, Shiga, Japan) and oligo $(dT)_{18}$ (Takara). Then the second round PCR was performed with the primers of 3'CDS, NUP and genes specific upstream primers.

Sequences and data analysis

PCR products were cloned into pMD18-T vector (Takara) and at least 3 recombinant plasmids were

Table 1. Primers used in Q-PCR analysis.

Name	Sequence (5′-3′)	Application
S6-F	TCTCGTTCCCCGCCACTG	S6 Q-PCR
S6-R	GCCCTTCCACTCATCACCCA	
S27a-F	GCGTGGTGGTGCTAAGAAGAG	S27a Q-PCR
S27a-R	AGTAATGACGGTCGAAGTGGC	
L29-F	GGTTGTCGTGCGCTTCG	S29 Q-PCR
L29-R	GCCGTTTCTGTGCCACTTAC	
L12-F	GGTCGGTGCCACTTCCTCA	L12 Q-PCR
L12-R	TCAATCGCTGCTTGCCTGTT	
S12-F	ACCGCACTCATCCACGACG	S12 Q-PCR
S12-F	GCATAGACCAACCCACTCACC	
S24-F	TGGTGTTCGTCTTTGGCTTCA	S24 Q-PCR
S24-R	ATGCCTCTAACTTTCTTCATTCTGTTC	
L27-F	TGGCAAGGTGGTGATGGTCC	L27 Q-PCR
L27-R	CCAGAGCGTGGCTGTAAGGAC	
GAPDH-F	GCCAGTCAGAACATTATCCCAGCCT	Internal control
GAPDH-R	GGTCCTCAGTGTATCCCAGAATGCC	

sequenced. Sequences were analyzed based on nucleotide and protein databases in the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Proteins structure and their function were defined by using NCBI (http://www.ncbi.nlm.nih.gov/structure). The protein topology prediction was performed using ProtParam at the ExPASy Bioinformatics Resource Portal (http://au.expasy.org/tools/protparam.html). Multiple sequences alignment was carried out using the CLUSTALW2.0 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), the Genbank accession numbers of randomly selected proteins were listed in Table 4. The sequence identities were calculated using GeneDoc software (http://www.psc.edu/biomed/). Phylogenetic tree was constructed by using the neighbor-joining (NJ) method with 100 bootstrap in the Mega 5 software package (Kumar *et al.*, 2004), and was present in a circle form.

RESULTS

cDNA and protein sequence analysis of the seven ribosomal protein genes in bighead carp

Seven RP cDNA fragments (RPS6, RPS12, RPS24, RPS27a, RPL12, RPL27 and RPL29) were isolated from the hepatic cDNA library through SSH. All seven full-length cDNAs were cloned. Some characteristics of these RP genes cDNA are listed in Table 2. In each full-length cDNA the 5'-UTR, 3'-UTR, ORF (open reading frame), poly (A) tail and the poly (A) signal sequence were present (Supplementary Fig. 1). The predicted molecular weight, isoelectric point, the number of α-helix and β-sheet in the predicted secondary structure, and the ratio (residues in a secondary structure to all residues) of each RP were listed in Table 3. The homology analysis of each bighead carp RP with other 10 species suggested that the amino acid sequences of RPs were well conserved (Supplementary Fig. 2 and Supplementary Fig. 3).

The expression of 3 RPs (RPS12, RPS24, RPL12) was repressed in the SSH cDNA library. The full-length of RPS12 cDNA (GenBank accession No. HM146123) was 518 bp including a 399 bp ORF. Based on NCBI conserved domains database, bighead carp S12 shared 99% identity with ribosomal L7Ae superfamily. The full-length cDNA of RPS24 (GenBank accession No. HM146122) was 531 bp including a 396 bp ORF. The 3D structure of bighead carp S24 shared 98% identity with ribosomal S24e superfamily. The full-length of RPL12 cDNA (GenBank accession No. HM146124) was 662 bp including a 498 bp ORF. The quaternary structure of bighead carp L12 was highly conserved (95%) with ribosomal L11 superfamily. 60S ribosomal protein L12, the eukaryotic counterpart of L11, shows three functional sites, 23s rRNA interface, L7/L12 interface, putative thiostrepton binding site.

The expression of the 4 other RPs (RPS6, RPS27a, RPL27, RPL29) was up-regulated in the hepatic cDNA library. The full-length of RPS6 cDNA (GenBank accession no. HM146127) was 875 bp including a 750 bp ORF. The full-length of RPS27a cDNA (GenBank accession no. HM146128) was 592 bp including a 471 bp ORF. Blasting bighead carp S27a in NCBI, UBQ superfamily and ribosomal S27 superfamily show high identity to the N-terminal and the C-terminal, respectively. The specific hit of the N-terminal part was ubiquitin (cd01803), including Ubq/RPL40e and Ubq/RPS27a fusions as well as homopolymeric multiubiquitin protein chains. The full-length of RPL27 cDNA was 526 bp (GenBank accession no. HM146125) including a 411 bp ORF. The structure of bighead carp L27 was similar to KOW motif of ribosomal L27e superfamily. The full-length of RPL29 cDNA (GenBank accession no. HM146126) was 417 bp and contained a 201 bp ORF. The structure of bighead carp L29 shared high identity with ribosomal L29e domain of ribosomal L29e superfamily.

Phylogenetic analysis of the seven ribosomal proteins

To analyze the seven RPs evolutionary relationship with other species, a NJ tree was constructed (Fig. 2). Each type of RP clustered together and was in a main branch, though they came from different species. In eve-

Table 2. The characteristics of identified ribosomal protein genes in bighead carp.

Gene name	Accession No.	Expression change in exposure to MCLR	Full-length of cDNA (bp)	Length of ORF (bp)
RPS12	HM146123	Down	518	399
RPS24	HM146122	Down	531	396
RPL12	HM146124	Down	662	498
RPS6	HM146127	Up	875	750
RPS27a	HM146128	Up	592	471
RPL27	HM146125	Up	526	411
RPL29	HM146126	Up	417	201

Table 3. The information of seven ribosomal proteins in bighead carp.

Protein name	MW (KD)	pI	Number of α-helices	Ratio (Residues in α-helices to all residues)	Number of β-sheets	Ratio (Residues in β-sheets to all residues)
S12	14.496	6.82	6	50.8%	7	26.5%
S24	15.186	10.27	5	32.5%	7	33.6%
L12	17.690	1.54	8	34.5%	5	22.4%
S6	28.867	10.96	7	43.37%	7	22.5%
S27a	17.999	9.68	3	19.2%	11	39.7%
L27	15.798	10.63	5	17.6%	7	36.8%
L29	7.670	12.03	3	72.7%	0	0%

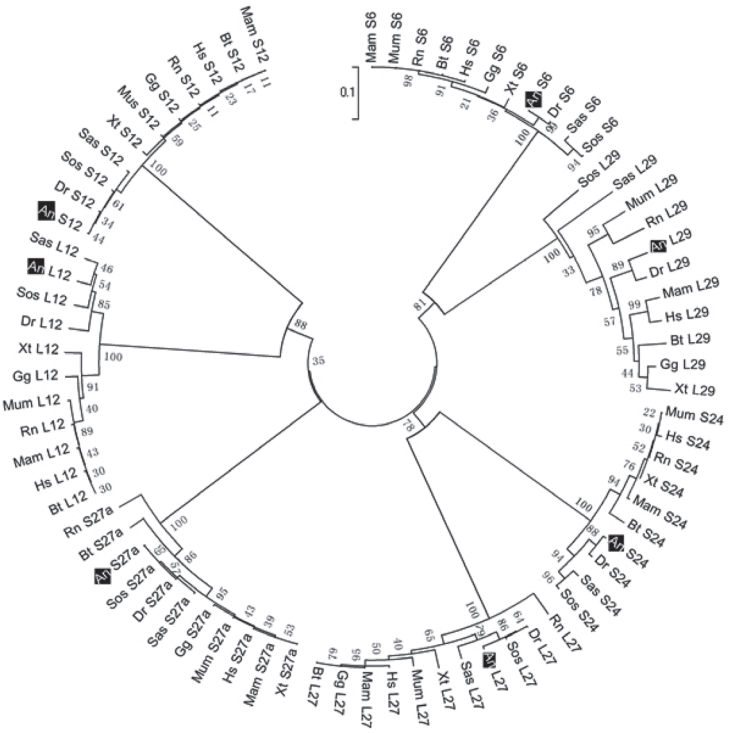

Fig. 2. Phylogenetic tree of seven ribosomal proteins from bighead carp and other species. Bootstrap values are indicated at the nodes (0.1 mutation per position). The GenBank accession numbers of sequences used are listed in Table 3. An, bighead carp (*Aristichthys nobilis*); Dr, zebrafish (*Danio rerio*); Sos, sole (*Solea senegalensis*); Sas, salmon (*Salmo salar*); Xt, xenopus (*Xenopus tropicalis*); Gg, chicken (*Gallus gallus*); Bt, cattle (*Bos taurus*); Mam, monkey (*Macaca mulatta*); Mum, mouse (*Mus musculus*); Rn, rat (*Rattus norvegicus*); Hs, human (*Homo sapiens*). Names of bighead carp proteins are noted with black background.

ry main branch, sequences from bighead carp, zebrafish, sole and salmon were closely related, and constituted a lower grade cluster. The evolutionary distances between the fish cluster and other vertebrates were different, but all the relationships were very close.

The mRNA expression profiles of ribosomal protein genes in vital organs

After bighead carps were injected i.p. with 200 μg MCLR per kg bw, the mRNA level of the seven RP genes were detected at 3 hr and 24 hr in vital tissues (liver, kidney, intestine, heart, spleen, gill) (Fig. 3). Three genes (PRS12, PRS24, PRL12) expression were repressed in

Table 4. The information of each selected ribosomal protein and its identity with bighead carp homology. Correspondences of common names with Latin names and GenBank accession numbers are shown.

Name	Species	S12		S24		L12		S6	
		Accession no.	Identity %	Accession no.	Identity %	Accession no.	Identity %	Accession no.	Identity %
Zebrafish	*Danio rerio*	NP_956340.1	99	CAX14783.1	99	AAT68131.1	94	NP_001003728.1	99
Xenopus	*Xenopus tropicalis*	NP_001008435.1	97	NP_001016625.1	91	NP_001007860.1	84	NP_001107495.1	99
Mouse	*Mus musculus*	XP_001480687.1	95	NP_997518.1	90	NP_033102.2	87	NP_033122.1	96
Sole	*Solea senegalensis*	BAF45900.1	98	BAF45912.1	96	BAF98659.1	93	BAF45894.1	96
Cattle	*Bos taurus*	BAC56364.1	96	XP_001249734.1	91	AAS20597.1	88	NP_001015548.1	96
Human	*Homo sapiens*	CAA37582.1	94	NP_001135757.1	90	BAD92708.1	87	AAH27620.1	96
Salmon	*Salmo salar*	ACH70842.1	98	ACN10478.1	95	ACH70859.1	95	ACH71012.1|	95
Monkey	*Macaca mulatta*	NP_001182594.1	96	Q4R5H5.1	95	NP_001180489.1	89	Q4R4K6.1	98
Rat	*Rattus norvegicus*	AAA42077.1	95	NP_112374.1	91	NP_001102668.1	88	NP_058856.1	96
Chicken	*Gallus gallus*	XP_419736.1	97	----	----	XP_415539.1	91	NP_990556.1	95

S27a		L29		L27	
Accession no.	Identity %	Accession no.	Identity %	Accession no.	Identity %
NP_956796.1	100	NP_001003434.1	94	NP_956018.1	99
NP_001016172.1	99	NP_001165147.1	83	NP_001016180.1	93
NP_077239.1	90	XP_001477526.1	79	XP_894555.1	89
BAF45917.1	100	BAF98680.1	77	BAF98677.1	99
XP_001788046.1	94	NP_001014862.1	85	AAI02314.1	95
NP_002945.1	99	EAW65182.1	85	BAG34735.1	94
ACH71005.1	99	ACI67687.1	77	ACI69670.1	96
XP_001087600.1	98	XP_001091393.1	85	XP_001108238.2	89
XP_001057026.1	98	XP_575373.1	83	XP_213135.1	92
NP_990284.1	98	NP_001165148.1	82	CAA40181.1	95

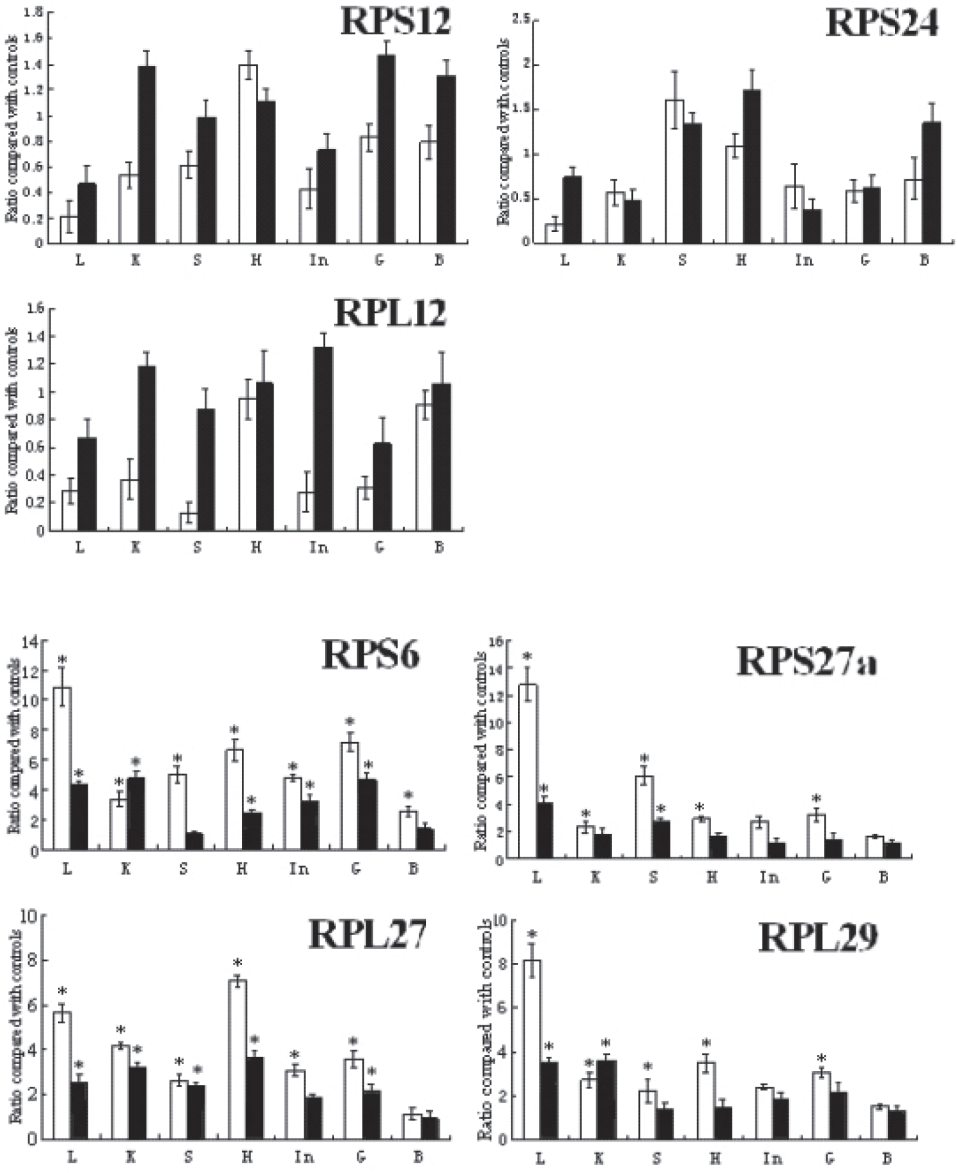

Fig. 3. Expression profiles of seven ribosomal protein genes in bighead carp at 3 hr (white column) and 24 hr (black column) post MCLR exposure. GAPDH was used as the internal control. Data are expressed as the mean ± S.D. (n = 3). The significant differences to the control ($p < 0.05$) are denoted with an asterisk (*). L: Liver; K: kidney; S: spleen; In: intestine; B: brain; H: heart; G: gill.

liver at 3hr post MCLR exposure. At 3 hr PRS12 mRNA level decreased in almost all tested tissues except heart, and the decrease degree was different. In heart there was a little increase of PRS12 mRNA level. At 24 hr PRS12 expression was down-regulated in liver and intestine; but a little increase was found in kidney, gill and brain; and in spleen no PRS12 expression change was detected. PRS24 expression decreased in almost all tested tissues at 3 hr, but in spleen and heart there was a little increase. At 24 hr PRS24 expression decreased in liver, kidney, intestine and gill; but expression increased a little in spleen, heart and brain. PRL12 expression decreased in all test-

ed tissues at 3 hr. At 24 hr PRL12 expression decreased in liver, spleen, heart, brain and gill, but increased slightly in kidney and intestine.

Expression of four genes (PRS6, PRS27a, PRL27, PRL29) increased obviously in liver. At 3 hr PRS6 expression increased significantly in almost all tested tissues. At 24 hr PRS6 expression also increased remarkably in almost all tissues, except a little increase in spleen and brain. PRS27a expression increased significantly in almost all tested tissues at 3 hr, and in liver the expression increased to about 13 fold ($p < 0.05$). At 24 hr PRS27a expression obviously increased in liver and spleen, a little increase was detected in the other tissues. PRL27 expression significantly increased in all tissues except in brain at both time points, only a little increase of PRL27 expression in brain was detected. PRL29 expression increased in all tested tissues at 3 hr. At 24 hr PRL29 expression also increased in all tissues, but the increase was not so significant compared to the increase at 3 hr.

DISCUSSION

In the past decades most attention was paid to the MCLR induced dysfunction in mitochondria, endoplasmic reticulum, and cytoskeleton etc. Ribosome is also an important organelle responsible for cellular metabolism in cells, whose structure and function are tightly connected with other cellular organelles, but the impairment of ribosomal structure and function by microcystins is still unknown. Though some reports have indicated that several organelles genes whose expression changed in response to MCLR treatment (Hao *et al.*, 2010; Zhao *et al.*, 2008; Li *et al.*, 2011), but no systematic information was available about MCLR toxicity on the expression of RP genes. In this study we identified cDNA of RP genes and investigated how MCLR affect their mRNA expression profiles in bighead carp.

There have been some *in vivo* studies on the toxic effects of MCs on the ultrastructures of liver and kidney in mouse and fish, such as swollen mitochondria, whirling of the rough ER, partial or total loss of ribosomes from vesicles, vacuolated cytoplasm, *et al.* (Li *et al.*, 2005; Zhao *et al.*, 2015). In one report the authors found expression change of 40S ribosomal protein SA and other proteins in response to MCLR in fish (Malécot *et al.*, 2009), and all the proteins were involved in protein synthesis and maturation, highlighting the role of organelles in protein synthesis and the complex cooperation associated with MCs toxicity.

Our data provided evidence that RPs were conserved in evolution. The seven cDNAs have typical eukaroytic cDNA features (Tran *et al.*, 2001), the start and stop codons, the typical poly (A) signal sequence and poly (A) tail in 3'-UTR, and share high identity with zebrafish counterparts. All these indicated that the cDNA sequences were from bighead carp. When the function of a protein serves a useful survival and a unique biological purpose, the selective pressure of evolutionary laws in nature conserves the protein sequence (Naftelberg, 2015). High sequence identities showed that the seven RPs were well conserved in bighead carp and other species, and indicated that these proteins might exert conserved functions. This result also gives us a clue to understand the role of RPs in the universal detoxicity mechanism in liver exposed to MCLR.

In this study the results of 3D structure blast showed that there were species-specific features of RP structures in bighead carp. Though their functions were conserved, the structures of RPs diverged in evolution (Goldstein, 2008). Only L12 had a specific hit to the known structure in conserved domain database blasting, the other six RPs were partially similar to some known domains, not exactly the same structures. The non-specific hits of each RP belonged to a functional related protein superfamily (Schmeing and Ramakrishnan, 2009). The pylogenetic analysis showed that the seven RPs were highly conserved. Bighead carp and 3 other fishes (zebrafish, sole, salmon) always closely clustered together in the NJ tree. This indicated that the evolution of the seven RPs was similar in the 4 fishes. Because there was evolutionary diversity of ribosomal proteins (Whittle and Krochko, 2009), the evolutionary distance between the 4 fishes and other selected vertebrates was different in every protein branch.

The RPs play an important role in ribosomal function. Expression of many proteins increased when bighead carps were exposed to MCLR (Li *et al.*, 2011), this indicated that MCLR-induced proteins increase, and this increase was the result of new proteins synthesis in cells. Hence, more ribosomes were required to translate the particular proteins to satisfy the cellular physiological change. Ribosomes from each species have obvious difference in size, composition and the ratio of protein to RNA, but the structure and function are well conserved throughout evolution (Ramakrishnan, 2011). It is difficult to determine the exact functions of each individual protein in ribosomes now, for most functions of RPs are still unclear, and the cooperation of all RPs is more important (Ben-Shem *et al.*, 2010). The synthesis and assembly process of ribosomes in eukaroytes involves the coordinated function of over 200 proteins (Fromont-Racine *et al.*, 2003). When bighead carps were exposed to MCLR,

different transcriptional change of RP genes in the same tissue appeared, this perhaps was caused by altered activity of RPs promoters induced by MCLR and different function of each RPs in anti-MCLR response.

In this study, the Q-PCR results of the seven RP genes expression in liver were consistent with the results of the suppression subtractive hybridization, indicating that the method is credible in gene selection. Because the dose of MCLR was high and consequently led to obvious acute toxicity in bighead carp (Li *et al.*, 2005), all seven genes' expression changed remarkably at 3 hr, and then went back to a moderately changed level at 24 hr. In bighead carp many proteins had to be synthesized immediately to against MCLR toxic effect, so ribosomes were required to produce new proteins quickly, and many RP genes expressed coordinately to assemble new ribosomes (Fromont-Racine *et al.*, 2003). The expression change of RP genes at 3 hr was notable. As time passed by, MCLR in bighead carp liver was transited to other metabolites or to other target organs, and the toxicity was eliminated at 24 hr. At this time too many ribosomes were unnecessary, and the expression of ribosomal protein genes was not so active as at 3 hr.

The different transcriptional change of the same RP genes in various tissues may be caused by MCLR distribution, metabolism dynamics and detoxification mechanism in bighead carp. Once MCLR was injected into bighead carp, a rapid transport of MCLR throughout the fish body takes place via the bloodstream and is distributed to various highly blood-irrigated organs or tissues (Li *et al.*, 2014). MCLR was accumulated in liver, intestine, kidney, gallbladder, gill, muscle, and brain. The cellular specificity and organotropism of MCs is due to the selective transport system, the specific bile acid transport system. MCs content was examined, the result showed that MCLR content was in the order liver > heart > blood > gonad > intestine = spleen = kidney = brain at 3 hr post MCLR exposure, and except in heart no detection in the other tissues at 24 hr post MCLR exposure (Lei *et al.*, 2008). Results from a subchronic toxicity experiment in sliver carp (Xie *et al.*, 2003) and a temporal dynamics study of MCs in bighead carp (Li *et al.*, 2007) suggested that these phytoplanktivorous fish may have a mechanism to degrade or bind MCLR actively after it enters the blood system, for no or very little MCs were detected in liver and other vital target organs after 3 hr post exposure (Martins and Vasconcelos, 2009).

In conclusion, in this paper we are the first to systematically identify and characterize RP geneswhich take part in anti-MCLR response in bighead carp. The amino acid sequences and structures of the seven RPs in bighead carp were conserved in evolution. The transcriptional expression profiles of the seven RP genes showed obvious acute intoxication in most tested organs in response to high dose of MCLR. It was the whole body coordinated system of RP genes expression across different tissues of bighead carp rather than any one gene expression from a single tissue that helped the fish to partly withstand MCLR induced toxic stress.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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