

## Molecular and Immunological Methods to Confirm Toxicity (Microcystin Production) of *Westiellopsis Prolifica* Isolated from Tigris River – Iraq

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### Abstract:

Several toxigenic cyanobacteria produce the cyanotoxin (microcystin). Being a health and environmental hazard, screening of water sources for the presence of microcystin is increasingly becoming a recommended environmental procedure in many countries of the world. This study was conducted to assess the ability of freshwater cyanobacterial species *Westiellopsis prolifica* to produce microcystins in Iraqi freshwaters via using molecular and immunological tools. The toxicity of *W. prolifica* was compared via laboratory experiments with other dominant bloom-forming cyanobacteria isolated from the Tigris River: *Microcystis aeruginosa*, *Chroococcus turigidus*, *Nostoc carneum*, and *Lyngbya* sp. significant expression of *mcyE* gene and microcystin production was most evident in *W. prolifica*. Contrary to the prevailing concept that *M. aeruginosa* is a main microcystin producer in freshwaters around the world, no significant microcystin production was observed with this species throughout the time points studied in our laboratory methods. As for *C. turigidus*, *N. carneum* and *Lyngbya* sp., neither *mcyE* expression nor microcystin production was significant. Data from *mcyE* expression by RT-qPCR were generally in agreement with those obtained from microcystin quantification by ELISA. Interestingly, *W. prolifica*, which showed clear microcystin-producing ability in this study and which was not reported before in the literature to produce microcystin, can be added as a new microcystin producer to the list of toxigenic cyanobacteria.

**Key words:** Cyanobacteria, ELISA, Microcystin, Tigris River, *Westiellopsis prolifica*.

### Introduction:

Around the world, freshwaters are considered as a main source of drinking water to the large number of peoples. Cyanobacterial blooms represent a nuisance to water environmental monitoring bodies for a number of reasons. In addition to the production of persistent unpleasant odors in waters that can deteriorate water quality (e.g., Geosmin), toxin production by cyanobacteria in surface waters poses a serious environmental and health concern due to its direct relation to both human and animal health (1, 2). Cyanobacteria are known to produce several types of toxins like microcystins, nodularins, cylindrospermopsin, anatoxins and saxitoxins (3, 4). Microcystins are the only toxins that have set standards by the World Health Organization in drinking and recreational water and are the only cyanotoxins screened for by municipal management agencies (5).

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A number of environmental factors, like hot and long summers and recurrent water pollution was lead to the formation of blooms. Several studies conducted to investigate water safety in a number of Middle Eastern countries confirmed the presence of cyanotoxins in common water sources. For example, toxigenic cyanobacteria and cyanotoxin production (microcystin and other toxins) were found in different Arabic freshwater sources from Tunisia (6), Egypt (7), Saudi Arabia (8) and Iraq (9, 10, 11, 12). However, despite the high incidence of water pollution and favorable environmental conditions for bloom formation in many Arabic countries, environmental and health authorities in these countries rarely consider cyanobacterial blooms as an environmental concern that necessitates mandatory periodic water testing (for possible toxin production) by water treatment stations and Iraq is no exception.

The Tigris, one of the two main rivers of Iraq, divides Baghdad into two parts: the eastern part is called Al-Risafa and the western part is called Al-Karkh. The river suffers from recurrent eutrophic events due to industrial and domestic discharge. Our previous work (10) in other parts of

Baghdad demonstrated the existence of the microcystin gene *mcyE* in *Westiellopsis prolifica* and other species using conventional PCR. To date, nothing is known about the toxigenicity of common bloom-forming cyanobacterial species in Iraqi freshwaters. Therefore, this study was designed to analyze and compare the toxigenicity of common bloom-forming cyanobacteria found in the Tigris. Our study is the first to clearly and conclusively document the in vitro microcystin production in *W. prolifica* (that belongs to the order Nostocales) using confirmatory molecular and immunological methods.

## Materials and Methods:

### Collection and preparation of samples

Water samples were collected from the main intake of the Tigris River from May to September 2014 from two geographically different locations (the first was located at North of Baghdad in Sader AlQanat whereas the second site was at the middle part in Al-Jadiryia area) where events of blooming were known to be commonly occurring in these locations. Phytoplankton samples were collected from the higher superficial layer with 20-30 cm deep from the intake of river in the amount of two samples, one for isolation solution and the second for cyanobacterial identification by using Lugol's solution was added to the collected water sample. The collection method was performed by using phytoplankton net 20 $\mu$  in mesh. Samples were transported to the lab directly and incubated under suitable and controlled conditions for algal growth (200  $\mu$ E/m<sup>2</sup>/s and 26 $\pm$  2 C°).

### Culturing of cyanobacteria and microscopic examination

The uni-algal culture of cyanobacterial species was obtained by using serial dilution method (13). Serial dilutions from the collected samples were prepared starting with 1ml of sample inoculated into 9 ml of BG11 nutrient solution. This procedure was repeated with examining each dilution until one species of algae was obtained. Uni-algal cultures were observed under microscope; the cell shape and size were monitored. The isolated phytoplankton were grown in sterilized BG-11 growth medium in 250 ml flasks. The cultures were incubated in a controlled-environment cabinet at 25  $\pm$  0.5 C° with cool white fluorescent lights ((200  $\mu$ E/m<sup>2</sup>/s and 26 $\pm$  2, 12 h light/12 h dark). Microscopic examination was performed on the glutaraldehyde-fixed samples using a Zeiss Axiovert 200 light microscope equipped with interference contrast. The morphological identification of cyanobacteria was done according to Desikachary (14). The optical

density was measured for initial inoculum of all isolated algae and unified to 0.02 O.D.

### DNA extraction from cultures of isolated cyanobacterial species

Genomic DNA was extracted from nearly 100 mg ww. for each cultured cyanobacterial species as follows: cells were lysed mechanically by bead beating with a Fast Prep 24 instrument (Mpbio) at speed 5.5 for 60 sec. The DNA was subsequently extracted using the Bio Ready\_Genomic DNA miniprep kit in accordance with the manufacturer's instructions (Miclub Biotech/China). Concentrations and purity of DNA were determined using a Nano drop ND-2000 spectrophotometer (Thermo scientific, USA) (15).

### Amplification of *mcyE* gene by PCR

Relying on the estimation of *mcyE* copy numbers, it is potential to quantify *mcyE* expression in cyanobacterial isolates by preparing external standards. These standards were prepared from the PCR products performed on the DNA samples from the cyanobacterial species using the primer pair HEPF 5'-TTTGGGGTAACTT TTTTGGGCATAGTC-3' and HEPR 5'-AATTCTTGAGGCTGTAAATCGGGTTT-3' (16). After several trials of optimization, the reaction mixture was carried out in 20 $\mu$ l including 5 $\mu$ l of PCR premix (Bionear, Korea), 2  $\mu$ l of DNA template and 1  $\mu$ l of each primer (2 picomole/  $\mu$ l), the volume was completed with distilled water. The PCR protocol was done in Master cycler gradient PCR (Eppendroff, Germany) involving one cycle for 2 min at 95 °C, 35 cycles for 90 sec at 95 °C, 90 sec at 59 °C, 60 sec at 72 °C and a final one cycle for 8 min at 72 °C.

### Subcloning of PCR products into pMID18-T vector

The PCR products from the amplification of *mcyE* using HEPF/R primer set were excised from the gel and purified using Biospin Gel Extraction kit (Biospin,China) according to the manufacturer's instructions. The DNA concentration was measured on the Nanodrop and the PCR fragments were subcloned into the TA cloning vector PMID18-T (TakaRaBiotechnology,China) by mixing the DNA insert/plasmid in a ratio of 4:1 and transformed into Cacl2-competent *E. coli* (DH5 $\alpha$ ). 10  $\mu$ l of the ligation mixture were used for transforming *E. coli* DH5 $\alpha$  via calcium chloride method. Positive transformants were screened using LB plates supplemented with 100  $\mu$ g/ml ampicillin. The plasmid DNA (*mcyE*-pMID18-T DNA) from the transformed *E.coli* cell was isolated using the Easy Pure plasmid miniprep kit™ (TransCan

Biotechnology, China) according to the manufacturer's instructions. After isolating the *mcyE*-pMID18-T DNA, the presence of the DNA inserted in the pMID18-T vector and its right sequencing of the vector using the Prism Big Dye cycle-sequencing system ABI 37030 DNA analyzer sequencer (Applied Biosystem, USA) (17).

### Primer design

The sequenced *mcyE* products from the Toxogenic cyanobacterial species were identified using BLAST/NCBI data and used to design a new primer pair (hema 2): hema2F 5'-TGGCGAGGAAGTAGGAACAG-3' / hema2R 5'-AATCGGGTTTACGGC TCTG-3'. Forq PCR and RT-qPCR experiments with the help of the Beacon designer software. The hema 2 primer set (ordered from Sangon Biotech, China) was tested for specificity by BLAST search.

### Preparation of standard curve

Quantitative real-time PCR assay (qPCR) was used for testing the newly designed primers (hema 2) and to prepare the absolute standard curve used for estimating *mcyE* gene copy number. In order to quantify the copy number of the *mcyE* genes, a ten-fold dilution series ( $10^2$ - $10^7$  copy/ $\mu$ l) from the linearized plasmid DNA (*mcyE*-PMD18-T vector) was generated by correlating the gene copy number and the threshold cycle (Ct) values (Figure. 2). The quantity of the target DNA (*mcyE*) was determined according to formula used by Vaitomaa *et al.* (18).

### RNA extraction

The RNA was extracted from cyanobacterial isolates at four different periods of growth (zero, one, two and three weeks) using the trizol plus kit (Miclub Biotech, China) according to the instructions of manufacturer's. Cell density was estimated at OD750nm. 1 ml from the unialgal culture from each cyanobacterial species was added to 250 ml of BG11 and this was considered as zero time. The total RNA was converted into cDNA using reverse-transcriptase kit (TaKaRa, Japan). The protocol of real-time PCR was carried out using a two-step process. Residual gDNA with RNA was destroyed with Dnase wipeout buffer prior to RT. A total of 100 ng of purified RNA was reverse transcribed using the QuantiTech® reverse transcription kit (Qiagen Inc.) following the kit manufacturer's protocol. The cDNA concentration and integrity were checked with ND1000 spectrophotometer.

### Analysis of gene (*mcyE*) expression by RT-qPCR

RT-qPCR was used for expression of *mcyE* gene. The thermal programme include: 2 min. at 50 °C, 3 min. at 95 °C, then 40 cycles of 15 sec. at 95 °C and 30 sec. at 57 °C, and 72 °C for two min. qPCR mixture was carried out in 25  $\mu$ l using SYBR green real time PCR mastermix (ToyoBo, Japan), 100 ng/ $\mu$ l of cDNA for gene expression assays and 1 pmol of each primer (hema 2). The reaction was carried out in ABI prism 7500 real time PCR (Applied biosystem, USA). Reactions were run at least twice with triplicate samples to ensure the reliability. The amplification efficiency was calculated as the average of all replicates. Melting curve analysis was performed after amplification steps to confirm the correct qPCR product and no non-specific products were formed (18).

### Analysis and quantification of microcystin by ELISA technique

The concentration of microcystin was determined intracellularly and extracellularly by indirect ELISA Kits from cyanobacterial species. Algal samples were prepared according to the method described by Lei *et al.* (19). Briefly, five isolates of cyanobacteria were cultured on BG11 nutrient solution and were filtered and harvested at first and second week of growth. The harvested cells (from the filtration) were broken by sonication for 10 min then the debris was removed by centrifugation at 10000xg for 10 min. The resulting supernatants were used for intracellular assessment of microcystin by ELISA kit (Enzo Life Science Inc., USA). As for the estimation of the concentration of the extracellular fraction of MC (that is released from cells into the culture medium), the filtrate (the liquid fraction from filtering the harvested cells), was used for extracellular assessment of microcystin. Microcystin concentrations were expressed as ng/ml.

### Statistical analysis

All data were analyzed using the SPSS-IBM program (Version-20). The least significant differences (LSD) were used to compare toxin quantities of multiple periods of growth for different cyanobacteria. Also, T-test and Mann-Whitney test analysis were used to analyze the role of the growth period on extracellular and intracellular toxin production while the frequencies were checked by applying Pearson test to estimate the relative correlation between quantities of toxin produced extracellularly and intracellularly and between quantities of toxin measured by RT-qPCR and ELISA assay. The significant differences were done under probability level ( $p \leq 0.05$ ).

**Results and Discussion:****Microscopic identification of cyanobacteria**

According to morphological characteristics of cyanobacteria mentioned in Desikachary (14), the

studied isolates were identified as *M. aeruginosa*, *C. turigidus*, *Lyngbya* sp., *W. prolifica* and *N. carneum* (Fig. 1).

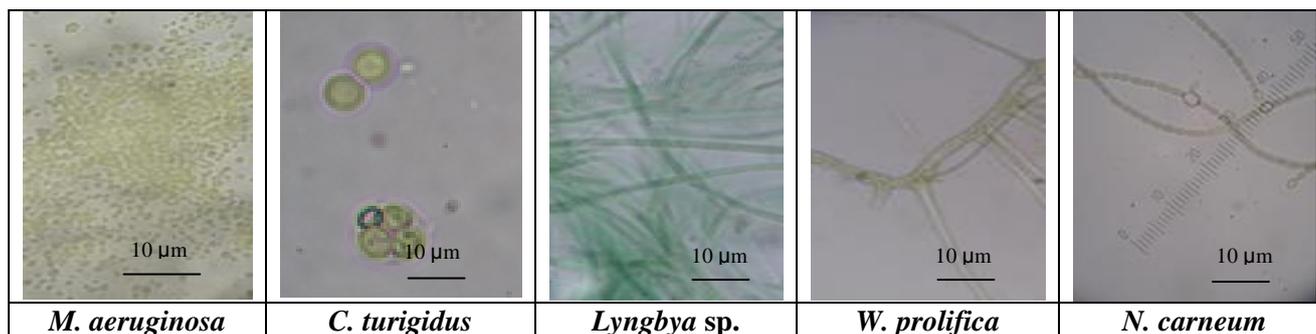


Figure 1. photomicrographs of the dominant algae isolated from Tigris River.

*Westiellopsis prolifica* Janet 1941

Eupri: Prokaryota

Kingdom: Ebacteria

Sub Kingdom: Negibacteria

Phylum: Cyanobacteria

Class: Cyanophyceae

Sub Class: Nostocophycidae

Order: Nostocales

Family: Hapalosiphonaceae

Genus: *Westiellopsis*

Species: *prolifica*

**New primer set 'hema 2' was used in qPCR and RT-qPCR experiments**

DNA was extracted from the five unialgal cultures of the dominant cyanobacterial species. All the tested species produced a PCR product of 472 bp that was detected in their DNA, which indicated that they harbored the *mcyE* (Fig.2).

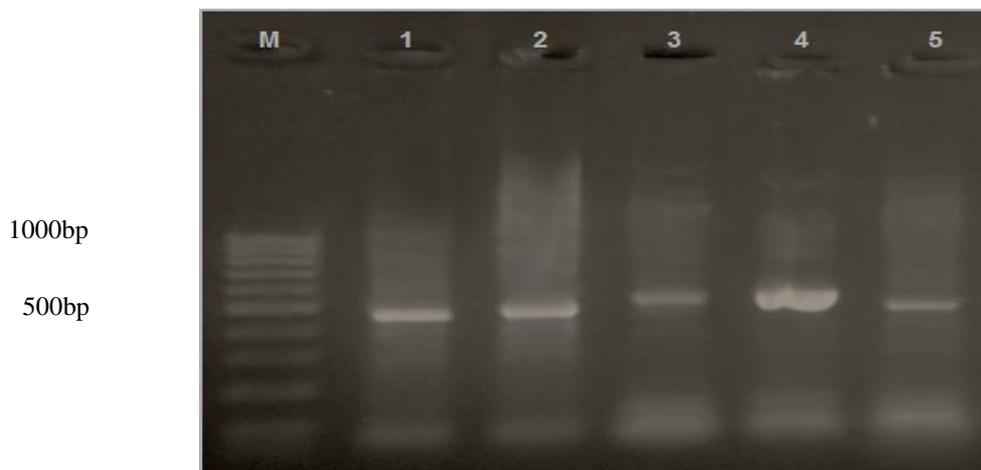


Figure 2. Gel electrophoresis of PCR-amplified microcystin gene *mcyE* (472bp) in cyanobacterial isolates. Agarose gel (1.5%) was run on 75 V/cm for 45 minutes, stained with ethidium bromide and visualized on a UV transilluminator. The figure shows results for 1-*M. aeruginosa*, 2- *C. turigidus*, 3- *Lyngbya* sp., 4-*W. prolifica* and 5- *N. carneum*

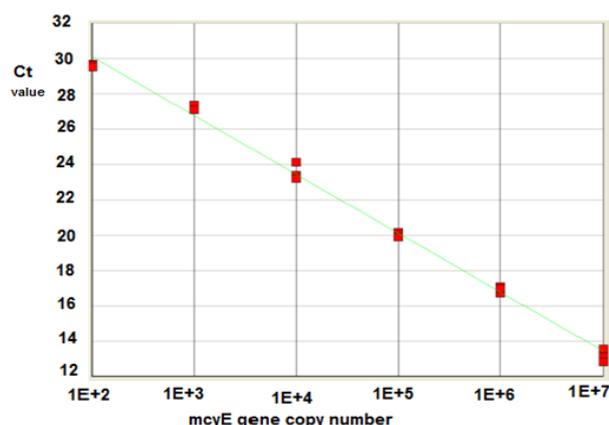
The PCR products amplified from *mcyE* (using HEPF/R primer) were sequenced, based on the DNA sequence; the new primer set (hema 2) was designed using the Beacon designer software. The BLAST search of the sequenced *mcyE* fragments, from all the five cyanobacterial species, exhibited high similarity to other *mcyE* sequences at the database, all sequences showed 100% identity except *Lyngbya* sp. which showed 99% to other sequences because the selected region was common in all studied algae's. These fragments (shared

among all the isolates studied) were chosen for designing hema 2 primer to give an amplicon of 102 bp from DNA/cDNA. hema 2 F/R primer showed high specificity for *mcyE* gene in studied isolates when searched via BLAST.

Furthermore, standard curve derived from the six serial dilutions ( $10^2$  - $10^7$  copy/ $\mu$ l) of the linearized plasmid DNA (*mcyE*-PMD18-T vector) using this set of primer yielded high efficiency reached 92% with high correlation coefficient ( $r = 0.99$ ) (Fig.3). Also, performing dissociation

analysis for hema2 primer showed non-specific PCR products or primer dimer and no signal was found in the negative control samples.

Hema 2 primers successfully amplified the target gene and quantified its copies. Therefore, designing primers from sequencing the *mcyE* region from the studied cyanobacteria provides a convenient tool for the real assessment of these cyanobacteria as mentioned in the study by Fortin *et al.* (20), which noted that primer design is a critical step in the development of qPCR for analysis of cyanobacteria.



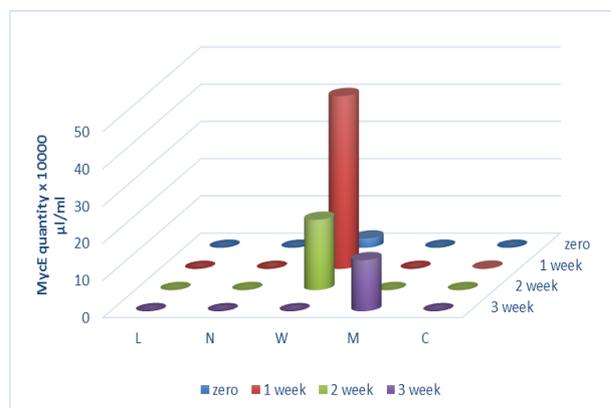
**Figure 3.** Amplification plot of standard curve prepared to quantify the copy number of the *mcyE*. X-axis shows the six serial concentrations ( $10^2$  - $10^7$  copy/ $\mu$ l) of linearized plasmid DNA (*mcyE*-PMD18-T vector) and Y-axis represents the cycle number. The curve yielded high efficiency (92%) with high correlation coefficient ( $r^2=0.99$ ).

#### Quantification of *mcyE* expression by RT-qPCR

The cDNA from studied organisms was successfully amplified using hema 2 primer, while negative controls did not produce any amplicon. RT-qPCR technique was used to quantify the expression of *mcyE* in the studied cyanobacteria relying on the estimation of the gene copy numbers that could be calculated from the standard curve. For *W. Prolifica*, there was highly significant expression of *mcyE* at zero time compared to the same time point with the other species. The expression increased significantly ( $P\leq 0.01$ ) at the 1<sup>st</sup> week (18.8 folds) but decreased notably at the 2<sup>nd</sup> and 3<sup>rd</sup> week ( $P\leq 0.01$ ) (Fig.4).

Interestingly, *W. prolifica* showed the highest expression level of *mcyE* when compared to the other studied cyanobacteria ( $P\leq 0.01$ ). On the other hand, although very little expression of *mcyE* was noticed in algal species *C. turigidus*, *N. carneum* and *Lyngbya* sp. At the time points studied (zero time, 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> week), the expression was non-significant differences ( $P\geq 0.05$ ) in all the time points of study. As for *M. aeruginosa*, there was non-significant expression of *mcyE* at zero

time, 1<sup>st</sup> and 2<sup>nd</sup> week. However, the expression increased significantly ( $P\leq 0.01$ ) at the 3<sup>rd</sup> week of growth (190 thousands folds increase) in comparison to the earlier times of the growth. There researchers investigated relationships between the proportion of *mcy*-genotypes in the population of cyanobacteria and the concentrations of microcystins in waters (21).



**Figure 4.** *McyE* expression from the five cyanobacterial species in different time points (zero, 1<sup>st</sup> week, 2<sup>nd</sup> week, and 3<sup>rd</sup> week). The quantity of *mcyE* (measured by Rt-qPCR) is expressed as copy/ $\mu$ l. L= *Lyngbya* sp., N= *N. carneum*, W= *W. prolifica*, M= *M. aeruginosa* and C=*C. turigidus*.

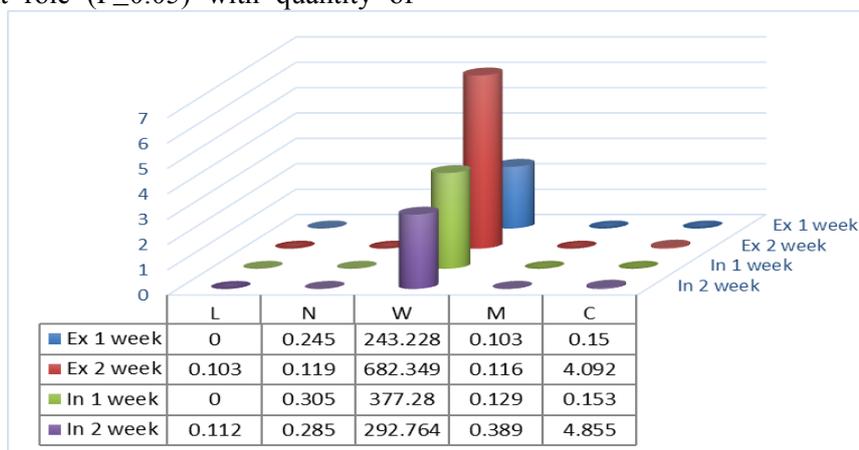
*W. prolifica* belongs to Nostocales, an order that includes species commonly found in tropical, subtropical and thermophilic habitats (22). In a recent study, several clusters of non-ribosomal peptide synthesis and polyketide biosynthesis have been found when mining the complete genome of *W. prolifica* which are essential for microcystins and nodularins synthesis (23).

#### Microcystin ELISA analysis

Extracellular and intracellular microcystin produced by studied cyanobacterial species during two weeks of growth were determined using ELISA assay. In general (with some exceptions), the quantity of the produced microcystin increased with the period of time. However, in all the species tested, except for *W. prolifica*, toxin production (whether intracellular or extracellular) was non-significant (Fig. 5). As for *W. prolifica*, toxin production (both intracellular and extracellular) was significant ( $P\leq 0.01$ ) compared with data from other species. Extracellular microcystin production increased from the first week from 243 ng/ml to 682 ng/ml on the second week. As for the intracellular microcystin production, on the first week it was 377 ng/ml and then it decreased on the second week to reach 292.76 ng/ml. Although decreased notably, the microcystin concentration on the 2<sup>nd</sup> week for

*W. prolifica* is still higher when compared with the 2<sup>nd</sup> week data from other species. Taken together, *W. prolifica* exhibited the most abundant microcystin production and this was in agreement with the gene expression data from RT-qPCR assay. The results showed that the studied growth periods had a significant role ( $P \leq 0.05$ ) with quantity of

microcystin produced for *W. prolifica*. There was a significant correlation between extracellular microcystin produced on the first and second week ( $P \leq 0.05$ ,  $r=0.9$ ), whereas this correlation was not significant for the intracellular microcystin production at the same period ( $P \geq 0.05$ ,  $r=0.7$ ).



**Figure 5. Quantification of microcystin production from the five cyanobacterial species in different time points (1<sup>st</sup> and 2<sup>nd</sup> week) by ELISA. Microcystin concentration was expressed as ng/ml. L= *Lyngbya* sp., N= *N. carneum*, W= *W. prolifica*, M= *M. aeruginosa*, C= *C. turigidus*. Ext= extracellular microcystin production and Int. = intracellular microcystin production.**

#### Correlation between *mcyE* gene and microcystin quantity

The two methods (ELISA and qPCR) displayed good correlation ( $P \leq 0.05$ ,  $r=0.9$ ) during the first week of growth with a decrease in correlation ( $P \geq 0.05$ ,  $r=0.6$ ) in the second week.

*M. aeruginosa*, which has been extensively studied by researchers, is a very common bloom-forming and important microcystin-producing species in freshwaters that exists all over the world. Data for *M. aeruginosa* were variable with significant microcystin expression detectable in the 3<sup>rd</sup> week (Fig. 4), while microcystin production (both intracellular and extracellular) gave non-significant differences at the studied periods of time for the toxin production assay (Fig. 5). Results about microcystin production studies can sometimes be conflicting because experiments performed under laboratory conditions can differ from those performed in natural environments. In several studies (20, 24, 25), *Microcystis* sp. produced more microcystins within the time period due to some factors like an increase in cell density with time, limitations in nutrient availability and other reasons. As indicated by Wood *et al.* (26), vicissitude in microcystins levels characterized by intermittent production of microcystin (bouts of up- and down-regulation in microcystin synthesis) could occur during time for some reasons such as changes in cyanobacterial cell density. Using a monoculture of *M. aeruginosa*, Ngwa *et al.* (27) reported continuous increase in microcystin concentration

from day 5 to day 21 while *mcyE* expression witnessed periodic fluctuations (vicissitude in gene expression: up-and-down regulation) during this period. By the same token, a study by Beversdorf *et al.* (28) indicated that *mcyE* abundance did not correlate with microcystin concentration. Thus, it is plausible to conclude that the lack of correlation seen sometimes between *mcy* expression and microcystin production could suggest that microcystin biosynthesis might be regulated post transcriptionally.

As for *C. turigidus*, *N. carneum*, and *Lyngbya* sp., although containing the microcystin gene (*mcyE*) as demonstrated by the PCR assay, both *mcyE* expression and microcystin production (intracellular and extracellular) were insignificant throughout all the studied periods of time. It is not surprising to see potentially toxigenic cyanobacteria (e.g., containing *mcy* gene) with no notable expression/production of microcystin. Earlier studies (29, 30) demonstrated that potentially toxigenic cyanobacteria (containing *mcy* gene) that did not produce microcystin can occur in natural environments. Several factors, among which nutrients, light, temperature, pH, CO<sub>2</sub>, competition (e.g., grazing) were studied extensively that could overall affect microcystin production. Still, microcystin production is a very complex process and till now factors triggering its production and why production can be variable between species or genotypes of the same species are not fully understood (26).

The alga *W. prolifica* is an understudied species in the literature especially in relation to microcystin production. Although Cirés *et al.* (22) studied *Westiellopsis* sp., neither *mcy* gene nor microcystin production was positive for *Westiellopsis* sp. Nonetheless, our previous work (10) showed the presence of *mcyE* in *W. prolifica* but till this date, none reported the ability of this alga to produce microcystins in vitro. The result of this study is the first to confirm the in-vitro microcystin production by this species. In addition, it is found this alga, has a high toxigenic potential (i.e., high microcystin-producing ability) that surpassed other bloom-forming species in microcystin production and was the main producer of microcystin. It should be mentioned that the level of the microcystin produced by *W. prolifica* in our study in-vitro assays and under laboratory conditions exceeds the recommended WHO guideline level (1 µg/l) set for this toxin in freshwaters (31). Thus, Iraqi environmental and health authorities should periodically monitor microcystin production and its concentration in Iraqi freshwaters and take the required countermeasures. As *W. prolifica* demonstrated high microcystin-producing ability, it would be interesting to study the *mcy* genes in more detail in this species.

### Conclusions:

This study is the first to clearly confirm the toxigenicity of *W. prolifica* by the production of *mcy E* transcripts and secretion of microcystin in vitro by molecular and immunological assays. *W. prolifica* show a high microcystin-producing ability and could be considered as one of the main microcystin-producing cyanobacteria in Iraqi freshwaters.

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### Conflicts of Interest: None.

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## طرق تقدير جزيئية ومناعية لتأكيد القابلية السمية (انتاج المايكروستين) من طحلب المعزول من مياه نهر دجلة – العراق *Westiellopsis prolifca*

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### الخلاصة:

تنتج العديد من الطحالب الخضر المزرققة السم الكبد المايكروستين. ولكون هذا السم يشكل خطراً على الصحة والبيئة، لذا فإن فحص مصادر المياه لوجود هذه السموم بصورة متزايدة يعد من الإجراءات البيئية الموصى بها في العديد من بلدان العالم. أجريت هذه الدراسة لتقييم الطحلب الأخضر المزرق *Westiellopsis prolifca* المعزول من نهر دجلة والذي شكل في الأونة الأخيرة ازدهارات في المياه العذبة العراقية وبيان قدرته على إنتاج المايكروستين عن طريق التحاليل الجزيئية والمناعية التأكيدية. وقد قورنت إنتاجية *W.prolifca* للسم عن طريق التجارب المختبرية، مع بعض الطحالب الخضر المزرققة السائدة في المياه العذبة العراقية والمعزولة من نهر دجلة وهي *Microcystis aeruginosa*, *Chroococcus turigidus*, *Nostoccarneum*, and *Lyngbya* sp. الأخصر *Microcystis aeruginosa* هو المنتج الرئيسي لسم المايكروستين في المياه العذبة في جميع انحاء العالم فإن إنتاج طحلب *W. prolifca* في هذه الدراسة قد فاق إنتاجية *M. aeruginosa*. أما الطحالب الأخرى المعزولة لم يلاحظ أي إنتاج لهذه الأنواع في جميع الفترات الزمنية التي لوحظت مختبرياً. أما بالنسبة *C. turigidus*, *N. carneum* و *Lyngbya* sp فلم يكن لها تعبير جيني ملحوظ للجين *mcyE* أو إنتاج ملحوظ لسم المايكروستين. بشكل عام فإن البيانات الناتجة من التعبير الجيني لجين *mcyE* بواسطة تقنية تفاعل سلسلة البلمرة اللحظي كانت متفقة مع تلك التي تم الحصول عليها من القياس بواسطة تقنية الامتزاز المناعي المرتب بالانزيم (ELISA). ومن المثير للاهتمام في هذه الدراسة، ان طحلب *W. prolifca* أظهر القدرة الواضحة لإنتاج سم المايكروستين، والتي لم يتم الإشارة عنها من قبل في الدراسات السابقة فيما يخص إنتاجه لهذا السم، ومن الممكن اضافته إلى قائمه الطحالب الخضر المزرققة كطحلب جديد منتج لسم المايكروستين.

الكلمات المفتاحية: طحالب خضر مزرققة، *Westiellopsis prolifca*، نهر دجلة، مايكروستين و ELISA.