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Detection of some cytotoxin genes in local isolate of *Oscillatoria* (Cyanophyta)

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ABSTRACT

The cyanobacteria, included *Ocsillatoria* can produce different types of toxin called cyanotoxins. which is a wide range of structurally diverse and biologically active in freshwater, marine and terrestrial ecosystems. This study aimed to detect the some cyanotoxin genes in local Oscillatoria species to know their able to produce toxins. The current study included the isolation and purification of seven local freshwater isolates of the genus *Oscillatoria* from Ramadi city, Anbar Governorate, western Iraq. that were detected microscopically, these species are: *O. acuta* , *O. princeps Vaucher* ,*O. annae* , *O. margaritifera* ,*O. proteus Skuja, O. sp.* ,*O. sancta*. the unialgal then axenic culture prepared using BG11 medium to get algal biomass. After the DNA extraction, by specific primers for some cyanotoxin genes with conventional PCR, was used to detect a group of selected genes, namely *mcy. E, ana.X, Ana.C*, *Ana. D* and *HEP*. The results showed the presence of these genes in local isolates, Which indicates the ability of the studied isolates to produce these types of toxins. The presented results indicate the potential threat to many organisms in the ecosystem, including animals and humans, as the results showed the presence of hepatoxin genes in five types of studied samples, and the presence of neurotoxins in four types of studied samples.

Introduction

Cyanobacteria (Blue Green Algae) are photosynthetic gram-negative bacterial common inhabitants of diverse aquatic freshwater, marine, and terrestrial environments. They are ancient prokaryotic life forms on Earth having photosynthesis which contributed oxygen to our atmosphere over 3.5 billion years ago, and can survive In different, sometimes frequently changing environmental conditions[1].

Cyanotoxin production Is considered to be an ancient trade Exceeding 2.5 billion years of age . Although cyanobacterial cell numbers change seasonally, [2].Blue green algae produce a wide range of toxic compounds, which are secondary metabolites, which are harmful compounds that pose a real danger. They have a toxic effect on many animals and humans. Some of these toxins reduce the ability of these organisms to produce new generations, and also reduce the growth rate of their individuals, while high concentrations of these toxins lead to their death [3].

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An algal bloom represents an overgrowth of cell cultures in response to elevated nutrient concentrations. Blue-green algae can affect the aquatic environment and human health through reduced water quality and production of cyanotoxins [4]. Flowering causes the rapid and sudden increase in the number of species or one type of algae in the water, and it occurs seasonally or during intermittent periods [5]. The reasons for the occurrence of this phenomenon are the availability of nutrients and other environmental factors such as the appropriate temperature and lighting for growth [6, 7].

Oscillatoria is a genus of filamentous cyanobacteria, commonly found in freshwater environments [8], *Oscillatoria* one of the oldest valid names among cyanobacterial genera, also one of the largest genera in described species richness, there are 629 species belong this genus, in local studies, there are 81 species listed in iraqi freshwater[9]. While there are several species within the genus *Oscillatoria*, it's important to note that not all species are toxic. However, certain species of *Oscillatoria* have been associated with the production of toxins that can be harmful to other organisms, including humans. One of

the well-known toxins produced by some *Oscillatoria* species is microcystin [10].

This study aimed to detect the some cyanotoxin genes in local *Oscillatoria* species to know their able to produce toxins.

MATERIALS AND METHODS

Site description and sample collection:

The isolates were collected from September to November 2022, from Ramadi city, Where it was washed with distilled water to remove mud and particles and some primer Algae Biomass was fractionated and each part was placed in test tubes It contains distilled water. Water was drawn by a micropipette and the process was repeated 9-10. Then, it was transferred to glass flasks and plastic boxes and incubated under laboratory conditions 20 ° C (±2) temperature, ventilation and lighting, After microscopic examination (40x)(figure :1), seven species of the genus *Oscillatoria* were obtained, after isolation, purification and identification, and then cultured on BG11 medium. For pure isolates.

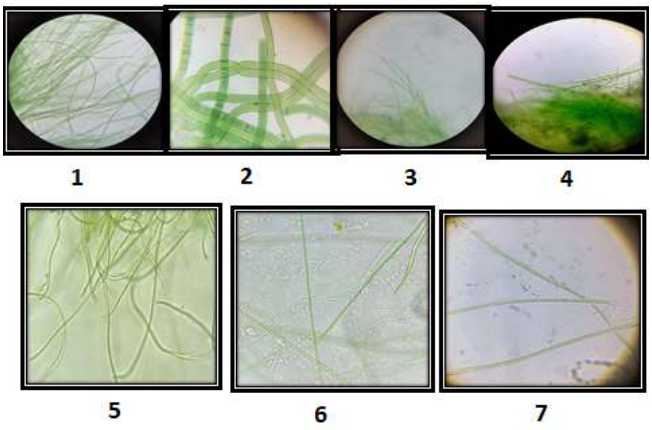

Figure 1: *Oscillatoria* specimens after microscopic examination to determine the species, 1.*Oscillatoria acuta,* 2. *Oscillatoria princeps Vaucher,* 3. *Oscillatoria annae ,* 4.*Oscillatoria margaritifera,* 5. *Oscillatoria proteus Skuja ,* 6. *Oscillatoria spp. ,* 7.*Oscillatoria Sancta.*

Genomic DNA extraction:

DNA was extracted from different types of blue-green algae using a genomic DNA extraction kit. Processed from Geneaid company and extracted according to the company's instructions.Molecular analysis PCR (Polymerase chain reaction)was used Various amplification reactions by PCR The PCR mixture was prepared In the PCR tubes supplied with the kit and the container on PCR components The other components were added to the reaction mix, DNA template 1 µL, ForwardPrimer 10 pmol (1 µL) , Reverse Primer 10pmol(1 µL) , and PCR water, the total became 20 µL. Amplification was performed in a mocycler Gene Amp 9700 (Applied Biosystems) with hybridization temperatures ranging from Between 44°C and 62°C.to search the size of the(mcy. E,Ana.C, ana.X ,and HEP) genes.

The reaction was performed according to the following denaturation program: initial denaturation at 94 °C for 5 min , and full denaturation At 94 °C for 1 min, annealing at 44 °C for the(mcy. E) gene. 48 °C for the (Ana.c) gene, and 54°C for the(Ana.x) gene. and 46 °C for the HEP Gene, 48°C the (ana. D) Gene, for 1 min,

extension at (72 °C) for 1 min, 35 cycles in total, and finally the final Elongation at (72°C) for 5 minutes , Amplified products were detected after 1% agarose gel electrophoresis. Contains ethidium bromide. A molecular weight marker (100 bp Smart Ladder Eurogentec) was used to assess magnitude of amplification Products [Table:1]

Primer		Primer sequenc5`→3`	Product Size(bp)	Tm (C°)	REFERENCES
AnaX	F	ATGGTCAGAG GTTTTACAAG	861bp	54°	[11]
	R	CGACTCTTAA TCATGCGATC			
AnaC	F	TCTGGTATTC AGTCCCCTCT AT	361bp	46° - 62°	[12]
	R	CCCAATAGCC TGTCATCAA			
AnaD	F	ACATAAACCT GCATTTTTAG GAG	458bp	46° - 62°	[11]
	R	AGGAACATAA GTTACTGAGG			
McyE	F	CCTGCACTCC CTGAGAGAAC	755bp	44°	[11]
	R	AATGACCGCC AATTTCAAAG			
HEP	F	TTTGGGGTTA ACTTTTTTGG GCATAGTC	472bp	46°	[13]
	R	AATTCTTGAG GCTGTAAATC GGGTTT			

RESULTS

This study was conducted to detect the presence of genes encoding toxins in some species belonging to the *Oscillatora*, and thus their ability to produce toxins and excrete them to their environment . the study depended on 7 species isolated from local freshwater, after identification, these species was : *O. acuta* , *O. princeps Vaucher*, *O. annae*, *O. margaritifera* ,*O. proteus Skuja* , *O. sp*., *O. sancta.* The genus *Oscillatoria* has been identified according to previous studies [14,15].

These identified species was mentioned in many different algal studies for local freshwater environments , in the same time, many researches mentioned to ability of *Oscillatoria* to produce some algal toxics [16].

The agarose gel electrophoresis for PCR products revealed owned species the toxic genes, the table 2 show results for this study , Neurotoxin genes were detected by using ana F and ana R primers to identify Species that are able to produce Anatoxin-D, Anatoxin-C, Anatoxin-X, The results showed of the current study are that not all studied species possess this Genes. The PCR products for the ana.X sample appeared in *O. proteus Skuja* and *O. sancta* with 861 bp moecular size (figure:2), The PCR products for the Ana.C Gene sample appeared in *O. princeps Vaucher* and *O.margaritifera* with molecular size 361 bp (figure:3). While there were no PCR product for the Ana.D gene, which showed that these types do not have these Genes so they do not produce these toxins (Table 2).

[Table 2]:Types of toxins produced by the studied species.

Gene	Size. (bp)	(C°) Tm	Sample OS1.	Sample OS2.	Sample OS3.	Sample OS4.	Sample OS5.	Sample OS6.	Sample OS7.
Ana. X	**861bp**	**54°**	_	_	_	_	+	_	+
Ana. C	**361bp**	**48°**	_	+	_	+	_	_	_
Ana. D	**368bp**	**46°**	_	_	_	_	_	_	_
Mcy. E	**766bp**	**44°**	_	+	_	_	+	_	_
HEP	**472bp**	**46°**	+	_	+	_	+	+	+

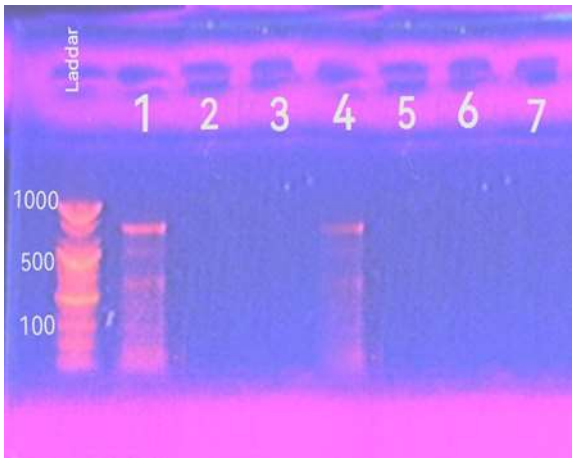

Figure 2: Agarose Gel Electrophoresis of PCR products for ana X gene of *Oscillatoria* species at a concentration of 1.2% , 100 V/cm for 60 min. with 100bp DNA ladder, *1-O. proteus Skuja , 2-O. acuta , 3- O. princeps Vaucher ,4- Oscillatoria sancta ,5- O. annae ,6- O. margaritifera ,7- Oscillatoria sp.*

Figure 3: Agarose Gel Electrophoresis of PCR products for Ana.C gene of *Oscillatoria* species at a concentration of 1.2%, 100 V/cm, for 60 min. with 100 bp DNA ladder. 1- *O. acuta* , 2- *O. princeps Vaucher* , 3- *O. margaritifera* , 4- *O. annae* , 5- *O proteus Skuja* , 6.*Oscillatoria sp*., 7- *O. sancta*.

Cyclic peptide genes were detected by using HEPF and HEPR primers, which is one of the types of hepatoxin To determine the species that are likely to produce Microcystin or Nodularin, the results of the current study showed that five of the studied species possess the HEP gene, which are *O. acuta*, *O. annae* , *O. proteus Skuja, Oscillatoria sp*. and *O. sancta*. The electrophoresis of the PCR products showed the appearance of bands of size 472 bp (figure 4).

Figure 4: Agarose Gel Electrophoresis of PCR products for HEP gene of *Oscillatoria* species at a concentration of 1.2% , 100 V/cm for 60 min. with 100bp DNA ladder, *1- O. margaritifera, 2- O. sancta, 3- Oscillatoria sp., 4 - O. proteus Skuja, 5- O princeps Vaucher, 6- O. annae, 7- O. acuta.*

Detection of the mcy E gene classifying a hepatoxin encoding the toxin Microcystin using specialized Primers mcyF and mcyR to determine the species that are likely to produce microcystin, and the results showed presence. For mcy. E for two of the seven samples, where two bands appeared *O. princeps Vaucher* and *O. proteus Skuja* with a size of 766 base pairs (figure 5).

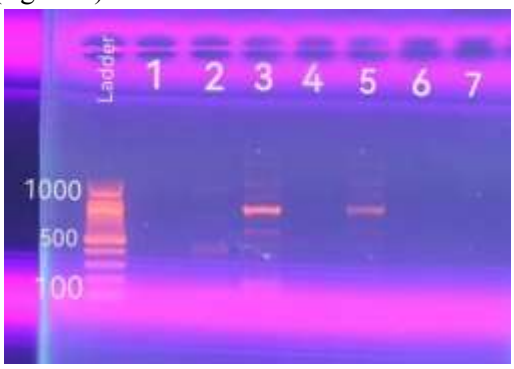

Figure 5: Agarose Gel Electrophoresis of PCR products for mcy E gene of *Oscillatoria* species at a concentration of 1.2% , 100 V/cm for 60 min. with 100bp DNA ladder, 1- *O. acuta ,2- O.annae ,3- O. princeps Vaucher, 4- O.margaritifera,5- O. proteus Skuja,6- Oscillatoria sp., 7- O. sancta.*

DISCUSSION

After presenting the results[Table 2], it was found that four of the five primers used in the study showed different results in the studied species. The ana D gene did not show any result of the seven types used in the study. *O. acuta*(os1), *O. annae* (os3) and *Oscillatoria sp* (os6) showed one type of hepatotoxin HEP and the other types did not show microcystin. *O. princeps Vaucher* (os2)showed two types of toxin production genes, one ana X Type of neurotoxin, mcy E is one type of hepatoxin . The species *O. margaritifera* (os4) showed one type of ana C neurotoxin and did not show hepatoxin genes. *O. proteus Skuja*(os5) showed three toxin-producing genes, one for producing the neurotoxins ana x, and two for producing the hepatotoxins mcy E and HEP. *O. Sancta* (os7) showed two types of microcystin production genes, the neurotoxin anaX and the hepatoxin HEP algal toxins produced by many types of algae such as Cyanophyta, Known toxic cyanobacteria. Toxic signs of these toxins are varied but involve mainly neuro- and hepatotoxicity. The majority of freshwater algal toxins are produced in water bloom. Toxic blooms of these cyanobacteria can be found in several types of water bodies. The main toxic genera are *Lyngbya*, *Anabaena*, and *Oscillatoria*. Toxins produced are hepatotoxic peptides or neurotoxic alkaloids. Human poisonings from algal toxins can be from ingestion of contaminated shellfish, fish, and drinking water or may be effects on physiology of fishes like effect of embryonic development of zebrafish ([17, 18].

Previous studies have indicated that the ability of *O. agardhii* to produce toxins may be linked to the growth of other types of algae, such as green algae, which stimulates them as a biological factor to produce toxins to inhibit the growth process of green algae as a type of allelopathic activity [19, 20]. The search of producing cytotoxins from local isolates of the *Oscillatoria* requires extensive studies because this species is widespread in local water bodies and has a great importance, in addition to the possibility of identifying the economic, medical or commercial importance of these toxins and producing them by the alga itself or through expression hosts using genetic engineering methods.

CONCLUSION:

This study is conclude the presence of cyanotoxins genes in some local oscillator species, the detection of MC-encoding genes in the biomass sample, the detection of several MC variants at low concentrations in water samples, and additional emphasis should be placed on MC detection. Gene coding Although the current MC variants have different toxicities, the results presented indicate the

potential threat to many organisms in the ecosystem including animals and humans.

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الكشف عن بعض جينات السموم الخلوية للطحلب *Oscillatoria* (Cyanophyta) المعزول من البيئة المحلية

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الخلاصة:

للطحالب الخضر المرزقة، بما في ذلك *Ocsillatoria*، القدرة على إنتاج أنواع مختلفة من السموم تسمى السموم الزرقاء. وهي عبارة عن مجموعة واسعة من السموم ذات التنوع الهيكلي والنشطة بيولوجيا في النظم البيئية للمياه العذبة والبحرية. هدفت هذه الدراسة إلى الكشف عن بعض جينات السيانوتوكسين في أنواع طحلب *Oscillatoria* المعزول من البيئة المحلية لمعرفة قدرتها على إنتاج السموم. تضمنت الدراسة الحالية عزل وتنقية سبع عزلات تعود للجنس *Oscillatoria* من مدينة الرمادي، محافظة الأنبار، غرب العراق. التي تم اكتشافها مجهريا، وهذه الأنواع هي: *O. acuta , O. princeps Vaucher ,O. annae , O. margaritifera ,O. proteus Skuja, O. sp. ,O. sancta*. تم تحضير المزارع احادية الخلية ثم المزارع النقية باستخدام وسط BG11 للحصول على الكتلة الحيوية للطحالب. تم بعدها استخلاص الحمض النووي، وبواسطة بادئات محددة لبعض جينات السيانوتوكسين وباستخدام تقنية تفاعل البلمرة المتسلسل PCR التقليدي، تم الكشف عن مجموعة من الجينات المختارة، وهي mcy E, ana X, Ana C, Ana D and HEP. وأظهرت النتائج وجود هذه الجينات في العزلات المحلية مما يدل على قدرة العزلات المدروسة على إنتاج هذا النوع من السموم. وتشير النتائج المعروضة إلى التهديد المحتمل للعديد من الكائنات الحية في النظام البيئي بما في ذلك الحيوانات والإنسان، حيث أظهرت النتائج وجود جينات السموم الكبدية في خمسة أنواع من العينات المدروسة، ووجود السموم العصبية في أربعة أنواع من العينات المدروسة.

الكلمة المفتاحية: *الطحالب الخضر المزرقة، طحالب الاوسلاتوريا، السموم الخلوية، ازدهار الطحالب.*