Detection of the Ability of some types of Cyanobacteria to The production of Antibiotics and Studying The Portability of These Antibiotics in The inhibition of Multidrug resistance Bacteria

Received : 7/12/2017 **Accepted : 18/1/2018**

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Abstract

The present study was carried out to detect the susceptibility of some types of cyanobacteria to the production of antibiotics and the ability of these antibiotics to inhibit the bacteria known for their multiple resistance to other antibiotics. Collected about 150 soil samples from five different governorates (Diwaniyah, Babel, Wasit, Baghdad and Basra), specifically from the soils near the Tigris and Euphrates rivers and for the period from 1/5/2016 to 1/5/2017. After implantation of the samples and sensitivity test using the disk diffusion method,the results yielded different inhibit- ion zones, ranging from 12.3 to 4.5, It also revealed the species of the growing cyanobacteria, which gave the largest inhibition zones and it was noted that most of them belong to three common types in Iraq *Microcystis aeruginosa, Anabaena variabilis* and *Hapalosiphon aureus*.In addition to 47 other species not disclosed in this study.

Keywords: Cyanobacteria, *Microcystis aeruginosa, Anabaena variabilis* and *Hapalosiphon aureus*

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Introduction

Photosynthesis bacteria are a group of bacteria that consume sunlight to release the energy needed to multiply, and their carbon source is carbon dioxide,it might be aerobic or an aerobic [1] . The phylum cyanobacteria is the oldest fossils that found in the earth their age is about 3.5 billion years. and it is the holy known photosynthesis bacteria , the also named blue- green algae as regarding to phycocyanine blue pigment[2].

Many species of cyanobacteria are pathogenic and may contain fatal toxins (Ian Stewart .,et al 2006), on the other side it contain nonpathogenic ,beneficial species(Bodas K .,et al 1995).these the last have been used in many experiments as Food supplement or in alternative medicine ,non-toxin produced

cyanobacteria also used in treatment of malnutrition[3], cancer and viral infections as considering them an active competent[4].

Cyanobacteria have been take under many different studies and their role in many ways , but its role in antibiotics production toke the little chance of these studies . the emergence of multi-drug resistant bacteria lead to the search for new antibiotics should continue as long as life continues.

the history of Benefit of Cyanobacteria in medicine is very ancient and belong to 1500 BC were *Nostoc spp* was used treat some diseases like Gout and fistula. But actual usage for this bacteria is start in 1900s by RE Moore and WH [5]. Cyanobacteria have many molecules that characterize with biological activity like antibacterial and antiviral activity ,where it contain polysaccharides, minerals, amino acid derivatives, carotenes and phenolic compounds ,as so as little concentrations of antioxidants [6].

Recently using Cyanobacteria in this field is of Noscomin from *Nostoc commune* which is active against *E coli*, *Bacillus cereus* and *Staphyllococcus epidermidis* [7].Bhatija *et al* 2006.found the antimicrobial activity of *Anabena spp* against Vancomycin resistance *Stahpyllococcus aureus* ,in 2007(Raveh, A. & Carmeli, S) isolated about nine different forms of Ambiguine I from *Fischerella* ,which showed antibiotic activity against *Bacillus subtlis* and *S albus* better than Strptomycin.[8].

Now a days studies about the ability of *Micrococcus lacustris* to produce many compounds and study its activity against many pathogenic bacteria like *S aureus* , *S epidermidis* ,*Salmonella typhi* , *Vibrio cholera* ,*Kelbsiela pneumonia*, *Bacillus subtlis*, *B cerus* and *E coli.*

In the present study we try to investigate the ability of many species of filamentous cyanobacteria to produce antibiotics and examining there effect against multi-drug resistant bacteria.

Material and methods

1. **Field of study and samples collections**

   150Samples were randomly collected from nearby tigers of the Tigris and Euphrates Rivers (Diwaniyah, Babel, Baghdad, Wasit and Basrah) , the number of samples according to the collection site are represented on table 1 below, during the period from 1/5/2016 to 1/5/2017. They were interrupted in combination due to rainfall to avoid their effect, The soil salinity was measured using the electrical conductivity meter and the pH measurement using pH meter.

   Table 1 the number of samples according to the collection site

The collection site	Number of samples
Diwaniyah	30
Babel	30
Baghdad	30
Wasit	20
Basrah	40

2. **Treatment and cultivation of samples**

The samples were transferred directly to the laboratory and 1 g of the sample was weighed and mixed with 10 g of PBS[1] solution in the test tubes and samples were cultured by usual methods by using 250

[1] Phosphate –Buffered Saline

ml Conical flasks contain 100 ml of [9]. With the incubator lighting using a fluorescent lamp, Carbon dioxide was added to the medium by 3% .The growth was observed for 10 to 30 days.

Subsequent planning and development of separate colonies were conducted under the same conditions described above for the purpose of obtaining pure colonies.

3. Preparation of cyanobactrial extract

Cyanobacterial isolate were concentrated by harvested them in stationary phase depending on using microcenterfuge at 5000rpm to 10 minutes and then washed with the same buffer used in medium . after then the samples mixed with ethanol and saved at 4ºC overnight. and then the samples were centrifuged at 5000rpm for 15 min [10].

4. examining of extract antimicrobial activity

Sensitivity test has been done by disk diffusion method against the bacteria mentioned in table 2 below , these bacteria characterize by multi_drug resistance isolated from the urine of patients in Diwaniyah Teaching Hospital,5mm filter papers have been saturated with20µL of the dried cyanobacterial extract. and putted in Nutrient agar petri dishes which already streaking with the multidrug resistance bacteria, the dishes incubated 24 h at 37ºC . The diameter of the inhibition zone was then measured [11].

Table 2 the multidrug resistance bacteria isolated from UTI patients used in the test

Bacteria	Ampicillin	Sulfamethoxasole+ Trimethioprim	Ciprofloxacin	Gentamicin	cefepime	Amikacin
Proteus	R	R	R	R	R	R
Pseudomonas	R	R	R	R	R	R
E coli	R	R	R	R	R	R
Staphylococcus	R	R	R	R	R	R
Klebsiella	R	R	R	R	R	R

5. Gene characterization of cyanobacteria

The isolates which gave the largest inhibition zone were subdued under gene characterization depending on PCR technique .

Firstly DNA extraction was done manually according to [12]. method ,the second step was made PCR experiment by using different primers belong to species *Microcystis aeruginosa, Anabaena variabilis* and *Hapalosiphon aureus*. (The most common species isolated in Tigris and Euphrates Rivers).were provided from Bionaire company(Korea).

These primers mixed with the extracted DNA and undergo the thermo cycler with programed at 95ºC for 3 min for denaturation,2 min at 55ºC for annealing and 3 min at read wave length of the bands.

Results

The current study was conducted on the soil surrounding the Tigris and Euphrates Rivers and five different governorates. The pH of all samples within the field of study was 7.5, The salinity rate of the soil was 17.5. As for soil temperature it ranged from 23-25ºC.

After the culturing of soil samples in the center mentioned, about 80 of 150 samples were give clear colonies, 30 of them grew in 10 days, while other 50 required 30 days for growth and emergence of clear colonies. most of the growing bacteria were developed with filamentous colonies with salinity levels up to 32%.the figure 1 showed the distribution of cyanobacteria in the site the study.

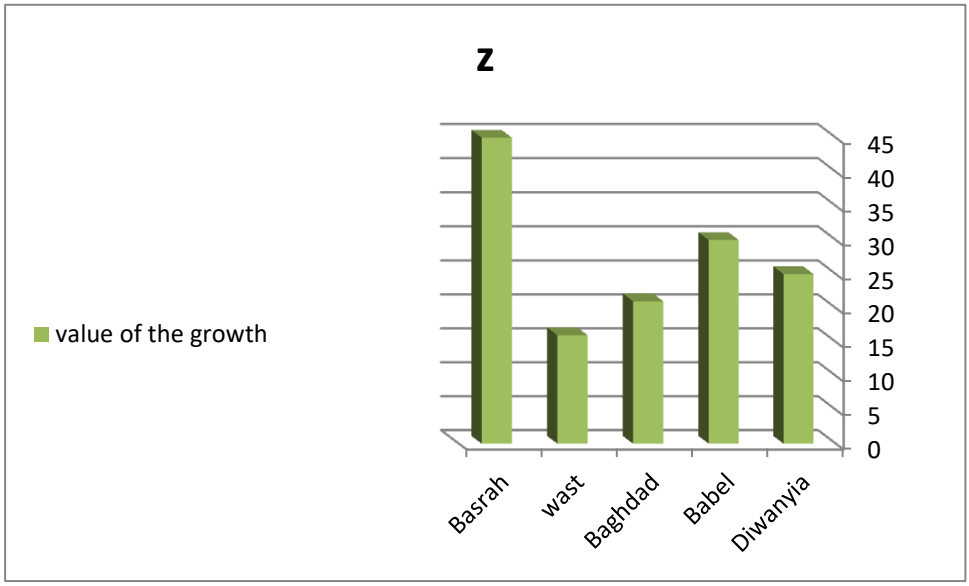


Fig 1 Distribution of the growth in the site of the study

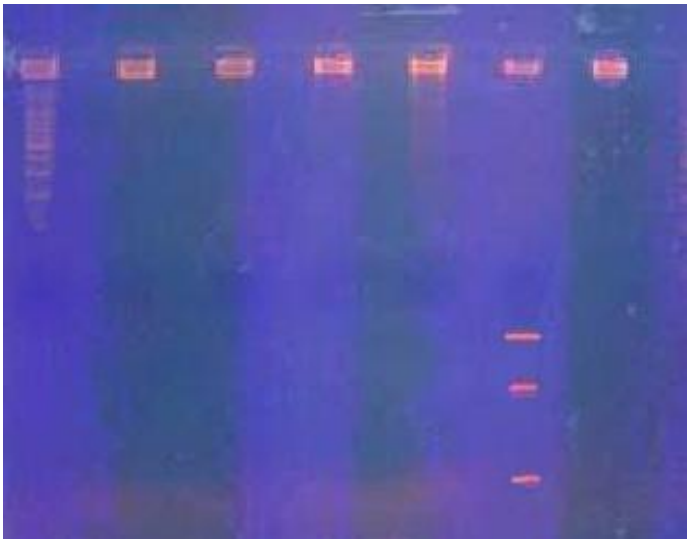
The sensitivity test on the group of bacteria listed in Table 2, which is characterized as multiple antibiotic resistance and 20 inhibition zones were 12.3 in diameter with constantly the period of the study against all types of bacteria. the antimicrobial activity results are listed in table 3
below.

Table 3 the frequency of Inhibition zones

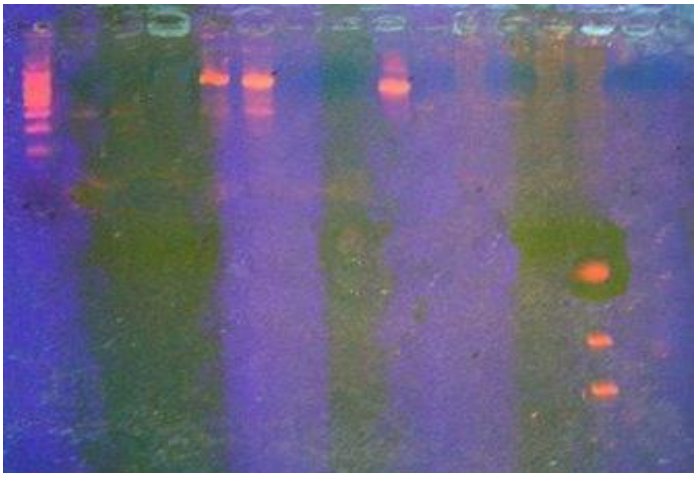
Inhibition Zone	Frequency
12.3	20
10.5	9
8.1	10
7.8	14
4.5	15

About 50 different isolates were obtained from the bacteria and PCR technique was done for only the species which gave a significant value of inhibition zone .and the primers provided for only three species of cyanobacteria depending on previous studies which referees that mostly common disturbed in the site of the study . And indeed the results were quietly similar to these studies i. e. *Microcystis aeruginosa, Anabaena variabilis* and *Hapalosiphon aureus*. Were positively founded in all soil samples at wave lengths 460,300.550 pbs respectively , beside the presence of another species need more studies and investigations.

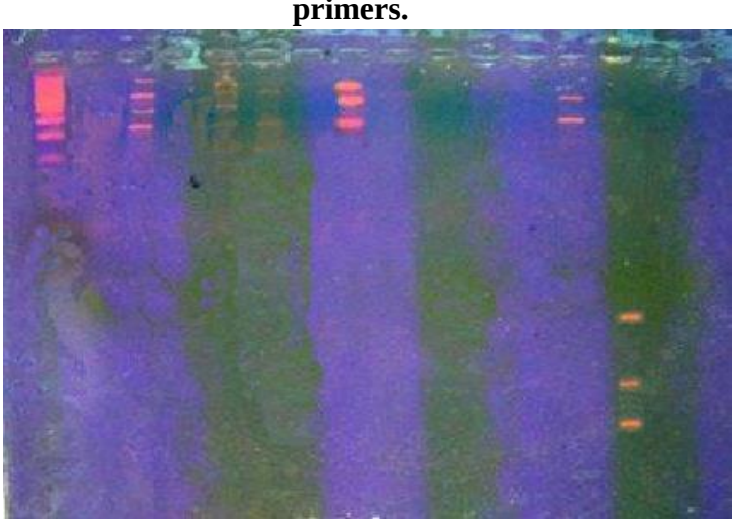
The pictures below shows the results of gel electrophoresis for the bands .



Pic 1The gel electrophoresis bands of end products by using *Microcystis aeruginosa* primers.



Pic 2The gel electrophoresis bands of end products by using *Anabaena variabilis* primers.



Pic 3 The gel electrophoresis bands of end products by using *Hapalosiphon aureus* primers

Discussion

The present study have been done to investigate the antimicrobial activity of many cyanobacterial soil spices and also to find out the ability of these antibiotics to inhibit multidrug resistance bacteria , samples have been inoculated on NASIII medium .the commonly used for isolation of cyanobacteria , the inoculated samples let for 30 days as incubation period in order to allow to slow growing bacteria to get enough time to make clear colonies , this way also help to ensure obtain correct suitable method can be used in the future . where in 2005 Anderson and Kawachi mentioned that the necessary period for incubation of cyanobacteria varying between few days (as regarding soil and fresh water types) and may reach to months (as regarding to oceans types). Therefore the incubation period can determined accurately the obligate halophilic species [13].

Nagle and Paul in (1998) through their studies to many groups of cyanobacteria recognized the presence of many secondary metabolic compounds produced from slow growing cyanobacteria as compared with the fast growing ones .thus we can depend the long time method as incubation way for cyanobacteria[14].

Also as important to know the present study find that 32%of saltinty was the optimum for producing antibiotics and there wasn't any production at high or low saltinty ratio than 32% so can conclude that fresh water is better source for new types of antimicrobial agents .

As regarding the effect of these agents and how inhibit bacteria need more studies in the future but we can benefit from Previous researches pointed that cyanobacteria can produce many compounds like macrolides compounds ,antitumor and antiviral compounds [15].

The final aim of our study was to characterize the species which produced the antimicrobial agents and the gene characterization was adopted because it more easy and fast and can give adequate results about the inoculated species. And as mentioned Previously above the PCR technique by using many primers for many common famous species in Tigris and Euphrates Rivers which mentioned in many studies in Iraq .and the results were acceptable with these studies beside the presence of another species which are never been isolated before in Iraq ,generally the mostly common species in the area were the much producers than un common ones . So we can conclude that our rivers have another fortune can be added to its fortunes and future studies most investigate about how to benefit of cyanobacteria in many fields as dietary supplement or antimicrobial agents or any another gainful compound.

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