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Diagnosis of a Local Isolate of Cyanobacteria (Fischerella Musciola) and Study of its Activating Efficacy against some Pathogenic Bacteria

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Abstract

The current study included isolating a local strain of cyanobacteria (*fischerella)* from the local environment (Mosul Dam Lake), and a molecular diagnosis was made for this strain to ensure its genetic classification and diagnosis. The exact scientific name for this strain is (*musciola fischerella)*. Different alcoholic extracts (organic extract, ethanolic extract, acetone extract) were prepared from a biomass of the studied isolates. Specific concentrations of these extracts were tested on pathogenic genera of bacteria isolated from local hospitals of Mosul city (*staph aureus, proteus mirabilis, klepsella pneumonia, Escherichia coli* and *pseudomonas aeruginosa*).

The results showed that the organic extract was the best regarding its ability to inhibit the growth of the studied pathogenic bacteria, as the diameter of the inhibition was (28) mm for bacteria (*staph aureus*) when using the concentration (250) µg/ml.

The ethanolic extract had less effect on pathogenic bacteria than the organic extract, and the maximum inhibition was (11) mm against the pathogenic bacteria *S.aureus*, (9) mm for *P.mirabilis*, (9) mm for *k.pneumonia*, (7) mm for *P.aeruginosa*, and (6) mm for *E.coli*, respectively, when using the ethanolic extract at a concentration of (250) µg/ml.

The acetone extract did not give any noticeable positive results, only at the concentration of (250) µg/ml, as the diameter of inhibition was (12, 11) mm for pathogenic bacteria (*S.aureus* and *P.mirabilis*) respectively, while (*k.pneumonia, E.coli* and *P.aeruginosa*) bacteria did not show any noticeable effect for all concentrations used of the acetone extract.

Introduction

Cyanobacteria, known as bluish-green algae, are aerobic prokaryotic organisms that are widespread in large parts of the world's environments. They are a precise engineering system that constitutes about (20-30) % of the production of oxygen gas. The extensive use of antibiotics has led to germs becoming more resistant because they have different resistance mechanisms [1]. Scientists have noticed that there are two types of resistance (natural or inherited and acquired), as Gram-negative bacilli are resistant to some antibiotics due to the presence of an exogenous membrane that controls the penetration of the antibody into the cell. The acquired resistance has begun to escalate than before, so it has become necessary to search for new materials to be used as a treatment. The natural product can be considered an effective compound that can be developed into a therapeutic material. The production of antimicrobials is also beneficial for the organism that produced it in its competition with another organism on food and place [2].

Cyanobacteria constitute a group of prokaryotic organisms that produce secondary metabolites that can be used in many fields such as energy, pharmacy and industries.

Antibiotics can be defined as compounds produced by microorganisms and have the ability to inhibit the growth of other microbes and to kill them. One of the riskiest epidemiological and

therapeutic phenomena to public health is the phenomenon of pathogen resistance to antibiotics [3]. Certain plasmids are found in pathogenic microbes with the ability to adapt with multi-drug resistance; it has become a major problem in treating human diseases, especially infectious ones, Germs can resist many antibiotics and have the ability to continue growing in the body taking the antibiotic, the thing that indicates that there are multi- resistant bacteria [4] and [5].

Therefore, the aim of the current study was to obtain a local isolate of cyanobacteria that produces toxic substances, molecularly diagnose them, and test their antibacterial activity.

Materials and Methods

Cyanobacteria used in the study (*Fischerella musciola*)

Method of Isolation:

The sample collected from the local environment of Mosul city was cultured under sterilization conditions on Chu10 solid medium that contains agar substance in Petri dishes and. A few drops of water sample brought from the designated area were dispersed, and the dishes were incubated in an incubator in continuous light conditions of (2500) Lux and a temperature of (28) °C for a period of (4-6) weeks, after which the growth of cyanobacteria colonies was observed. Each colony is transferred individually onto a Petri dish containing solidified Chu10. The culture was then transferred from Petri dishes to Chu10 liquid medium in (250) ml glass beakers containing (100) ml of medium. The beakers were then placed in a shaker incubator under sterilization conditions and with a shaking speed of (100) cycles/per minute, illumination intensity of (2500) lux, and temperature (28) °C until the appropriate growth is obtained in order to obtain a pure isolation free of any other microbial contaminants such as fungi and bacteria [6].

Methods of Analysis:

1- Measurement of final pH:

After the incubation period for each experiment, the acidity function of each beaker was measured.

2- Measuring the biomass of cyanobacteria:

After the end of the incubation period, a certain size is taken from the culture and filtered by means of filtering paper (type Watmon No1) of a known weight. The filtering paper containing the cyanobacteria cells is left for (24) hours to dry and its weight is measured by a sensitive scale. The difference between the two weights is the weight of the cyanobacteria biomass.

3- Chlorophyll pigment measurement:

Chlorophyll pigment was measured using method [7].

4- Measurement of total protein:

The total protein content was measured using method [8]; the Follin reagent was used, and BSA (Blood Serum Albumin) was used as a standard solution for drawing the standard curve**.**

5- Carbohydrate content determination:

The carbohydrate content of the cyanobacteria used in the study was estimated; the method described in [9] was adopted.

Isolation of DNA (Deoxyribo Nucleic Acid):

The first step that researchers specialized in genetic engineering take is the isolation and purification of DNA.

Genomic deoxyribonucleic acid extraction:

Tools provided by (Wizard (R) Genomic DNA Purification kit) were used which included the following solutions:

(Nucleliysis solution, RNAse solution, protein precipitation solution, DNA Rehydration solution, isopropanol and ethanol (70) %), as described in [10 and 11]

Estimating the concentration and purity of the extracted DNA:

The DNA concentration was determined by estimating the UV absorbance using a (SPECTRO Photometer) and the wavelength (260) nm as described in [12]. DNA samples were taken from several types of cyanobacteria that were studied for the (RAPD) test; work tools used were equipped with Indicators of polymorphic random interaction of the DNA sequence DCR:

(Accu power PCR Pre Mix Which Covered by Koreapat No.162) as described in [13]

Random primers used in the study:

The following primes were used as shown in the table:

primer	Sequence	
forward	5′- GGGGAATYTTCCGCAATGGG-3′	
Revers	5′- GTTGCGTCTGTGTGCCTAGG-T-3′	

Preparation of Extracts

After growing cyanobacteria for (20) days in the previously prepared photo reactor under the optimum culture conditions selected from previous experiments, and the biomass was precipitated by centrifuge to obtain algal cells, the filtrate was neglected and the precipitate was taken and placed in an electric oven at a temperature of (40)°C for drying. After that, the precipitate is dissolved using a solvent with crushing and breaking the cells using a ceramic mortar for two hours with the addition of liquid nitrogen.

The extract was placed in the incubator shaker at a temperature of (28) °C for a period of (3) days. After the 3 days, the extract is centrifuged to obtain the filtrate and remove the precipitate, and then the filtrate was put in a glass beaker at a temperature of (40) °C for (18) hours for drying, then (1) one gram of the extract is weighed and dissolved in (5) ml of DMSO if the solvent was organic or alcoholic. The prepared solution is sterilized by pasteurization at a temperature of (65) °C for (15) minutes while performing the required dilutions. Several concentrations of each extract (organic, ethanol, acetone) were prepared as follows: (50, 100, 150, 200, and 250) µg/ml [14 and 15].

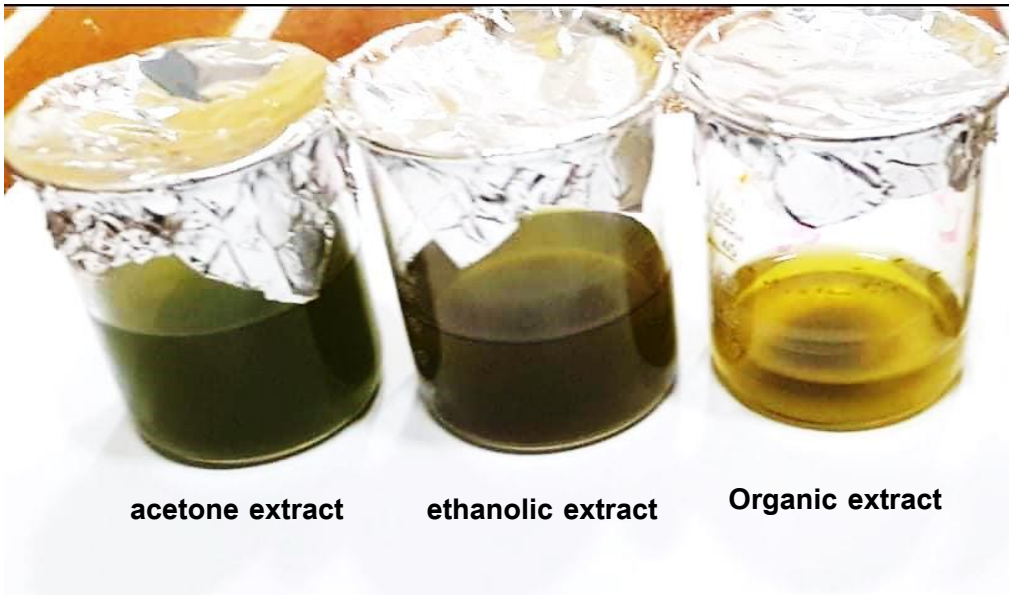


Figure (1), Prepared Extractions

Collection of Bacteria Samples:

pathogenic bacteria samples were brought during the study period from the laboratories of pathological analyses of Al-Salam Hospital (Al-Wahda neighborhood) and the laboratories of pathological analyses in Al-Zahrawi Hospital (Al-Masarif neighborhood), and they were diagnosed in the laboratory and chemically.

Results and Discussion

Microscopic diagnosis of cyanobacteria isolation:

Colonies with a bluish-green color were isolated and examined microscopically by a compound microscope and diagnosis was made. A pure isolate of Cyanobacteria (*Fischerella musciola*) was obtained. This genus is characterized by its vegetative structure, which is in the form of branched filaments, and the lateral branches are relatively short. The vegetative cells are permeated with heterogeneous vesicles depending on the references [4].

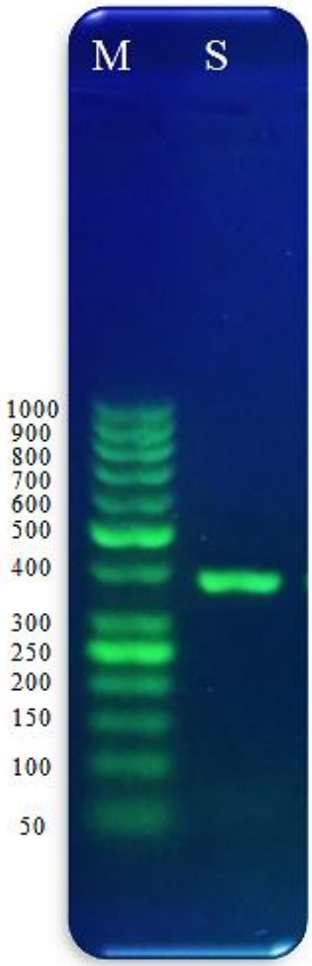
Molecular diagnosis of cyanobacteria:

DNA isolation:

The DNA of Cyanobacteria (*Fischerella musciola*) was extracted; the order of the nitrogenous bases and the PCR reaction products were examined with the primers of the resulting bundles, and the order and organization of the genes documented in the National Center for Biotechnology Information (NCBI) were examined.

PCR reaction output:

After electrophoresis of the gel, the size of the bundle appeared with a reaction product of (400) base pairs, as in the figure:



Sequencing reaction output:

A similarity ratio of the strain (Fischerella musciola SAG 1427.1) appeared in the gene bank according to the following figures, the results of the analysis of the sequences of nitrogenous bases for the studied isolate (SAG 1427.1) appeared as follows:

ACCACCGTGCTGCACTTAGCAGCAGAAAGAGGTGCAGTAGAAGACTTAGAACTCGAAGAA 103
GTAATGTTAAAAGGTTTCCGTGATGTTAAATGCGTGGAATCTGGTGGTCCTGAACCTGGT 163
GTAGGTTGTGCTGGTCGTGGTATCATCACCGCCATTAACTTCTTGGAAGAAAACGGTGCT 223
TACCAAGACTTAGACTTCGTATCATACGACGTATTGGGTGACGTTGTCTGTGGTGGTTTC 283
GCTATGCCTATTCGTGAAGGTAAAGCACAAGAAATCTACATCGTTACCTCTGGAGAAATG 343
ATGGC 348

With the control strain (832456.1K), and the percentage of convergence between the local strain and the control strain was up to (98)%, as there were sites for replacement between the two strains, three of which were transformative at sites (133, 154, 193) and the last three were reciprocal at sites (223, 271, 337).) the reciprocal sites had no effect on the genetic code; neither on the amino acid responsible for its formation.

Fischerella muscicola SAG 1427-1 = PCC 73103 clone HAB02 gene, partial cds

Fischerella muscicola SAG 1427-1 = PCC 73103 clone HAB02 gene, partial cds
Sequence ID: KT832456.1Length: 349Number of Matches: 1
Range 1: 44 to 348GenBankGraphicsNext MatchPrevious Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
524 bits(580)	9e-145	299/305(98%)	0/305(0%)	Plus/Plus

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Query  1    ACCACCGTGCTGCACTTAGCAGCAGAAAGAGGTGCAGTAGAAGACTTAGAACTCGAAGAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  44   ACCACCGTGCTGCACTTAGCAGCAGAAAGAGGTGCAGTAGAAGACTTAGAACTCGAAGAA  103

Query  61   GTAATGTTAAAAGGTTTCCGTGATGTTAAGTGCGTGGAATCTGGTGGTCCCGAACCTGGT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  104  GTAATGTTAAAAGGTTTCCGTGATGTTAAATGCGTGGAATCTGGTGGTCCTGAACCTGGT  163

Query  121  GTAGGTTGTGCTGGTCGTGGTATCATCACTGCCATTAACTTCTTGGAAGAAAACGGTGCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  164  GTAGGTTGTGCTGGTCGTGGTATCATCACCGCCATTAACTTCTTGGAAGAAAACGGTGCT  223

Query  181  TACCAAGACTTAGACTTCGTATCATACGACGTATTGGGTGACGTTGTGTGTGGTGGTTTC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  224  TACCAAGACTTAGACTTCGTATCATACGACGTATTGGGTGACGTTGTCTGTGGTGGTTTC  283

Query  241  GCTATGCCTATTCGTGAAGGTAAAGCACAAGAAATCTACATCGTTACCTCTGGTGAAATG  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  284  GCTATGCCTATTCGTGAAGGTAAAGCACAAGAAATCTACATCGTTACCTCTGGAGAAATG  343

Query  301  ATGGC  305
            |||||
Sbjct  344  ATGGC  348
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Figure (2) Matches of the order of the nitrogenous bases of DNA isolate *Fischerella musciola SAG 1427.1*

Inhibitory efficacy of Cyanobacteria extracts against the studied bacteria:

The results showed, Table (1), that the organic extract of cyanobacteria showed a clear activity against all types of the studied pathogenic bacteria. The maximum inhibition diameter was (28) mm when using a concentration of (250) µg/mm of the organic extract against *S.aureus* bacteria, while the other bacterial genera were less affected. The reason is due to the fact that the organic extract includes various raw chemical compounds that have high inhibitory efficacy such as alkaloids, steroids, and pigments, as well as the toxins contained in cyanobacteria in general [16 and 17].

The effective impact of this extract against the pathogenic bacteria studied is due to the effect of the active compounds against the mechanisms of biological resistance of pathogenic bacteria in general, regardless of the virulence factors of each type of them. The inhibitory effect of the organic extract may be due to the presence of cyanobacteria toxins which may inhibit the

synthesis of proteins necessary for the biosynthesis of virulence factors for the studied pathogenic bacteria [18], and the results of the current study are in agreement with the study of [19].

The results of the current study also demonstrated that the ethanolic extract showed significantly less inhibitory activity than the organic extract against the studied pathogenic bacteria; when using the concentration (250) µg/ml, the diameter of the inhibition was (11) mm for the bacteria (*S.aureus*), while the diameter of the inhibition was (9) mm for bacteria (*Proteus Mirabilis*), (9) mm for bacteria (*Klepsella Pneumonia*), (7) mm for bacteria (*Pseudomonas aeruginosa*), and (6) mm for bacteria (*E.coli*).

[20] stated that the ethanolic extract of the cyanobacteria *F. musciola* contains a number of alkaloid compounds such as (1-propene, 3-C2-cyclopentenyl-2-methyl-1, di phenyl), in addition to the compounds (3, 6-Nonadien) (2-Amino-1, propandiol), and it had a negative effect on pathogenic bacteria. It was also noted that the ethanolic extract of cyanobacteria (*Gleocapsa*) contains a number of compounds (Hydrazine, 1, 1-dimethyl, Eicosane, octacane); no positive effect of this extract against the studied pathogenic bacteria was observed. The results obtained in the current study are close to the results of a number of studies related to cyanobacteria extracts [21].

The results showed that the acetone extract did not show a clear effect against the studied pathogenic bacteria, as the studied bacteria showed a clear resistance to the extract and showed no sensitivity towards it except at the concentration (250) µg/ml for (*S.aureus*) and (*Proteus Mirabilis*) as the diameter of activation reached (12, 11) mm respectively, which indicates the clear resistance of the studied bacteria to the concentrated active compounds contained in this extract.

[22] indicated that acetone extract of the genera (*Chorella, Fischerella* and *Gleocapsa*) had a clear effect on the growth of pathogenic bacteria, and indicated that the raw extract contained a number of active compounds, and it was noted that the raw extract of *Chlorella algae* contains the compounds (Cyclopentane, methyl-n. -Hexane) which would have relatively little effect against pathogenic bacteria.

Table (1)

Effect of extracts prepared from Cyanobacteria (Fischerella musciola) on the studied pathogenic bacteria for the diameter of the inhibition circle measured in mm)

Type of Bacteria	Organic Extract (µg/ml)					Ethanolic Extract (µg/ml)					Acetone Extract (µg/ml)				
	50	**100**	**150**	**200**	**250**	**50**	**100**	**150**	**200**	**250**	**50**	**100**	**150**	**200**	**250**
Staph aureus	- (0.01)	12 (0.09)	18 (0.80)	22 (0.90)	28 (0.35)	- (0.00)	- (0.11)	5 (0.11)	8 (0.01)	11 (0.09)	- (0.00)	- (0.00)	- (0.00)	- (0.00)	12 (0.01)
Proteus mirabilis	- (0.00)	10 (0.90)	17 (0.90)	21 (0.30)	25 (0.01)	- (0.00)	- (0.00)	4 (0.30)	6 (0.35)	9 (0.08)	- (0.00)	- (0.10)	- (0.00)	- (0.00)	11 (0.85)
Klepsella pneumonia	- (0.00)	9 (0.10)	15 (0.11)	18 (0.15)	22 (0.11)	- (0.00)	- (0.00)	5 (0.45)	7 (0.09)	9 (0.70)	- (0.01)	- (0.00)	- (0.00)	- (0.00)	- (0.00)
Pseudomonas aeruginosa	- (0.00)	8 (0.11)	12 (0.10)	16 (0.09)	21 (0.19)	- (0.01)	- (0.00)	3 (0.11)	5 (0.11)	7 (0.18)	- (0.09)	- (0.00)	- (0.00)	- (0.00)	(0.00)-
Escherichia coli	- (0.00)	8 (0.10)	14 (0.90)	16 (0.11)	18 (0.90)	- (0.00)	- (0.00)	2 (0.19)	5 (0.35)	6 (0.09)	- (0.00)	- (0.00)	- (0.00)	- (0.00)	- (0.00)

*** Each value is the average of three repeatitions and the numbers between brackets represent standard deviation**

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