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Molecular Identification of Locally Isolated Bacteria from Soil in Thiqar Governorate and Ability to Produced Antibiotics

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Abstract

The objective of this study was to isolate and diagnose local bacterial isolates produced antimicrobials from some soil in Thiqar governorate and detecting the presence of 16SrRNA gene using DNA Sequence technique. The study included collected 25 soil samples from different regions of Thiqar Governorate, 13 bacterial isolates wasisolated depending on shapes and colors, RL1 and RL2.These isolates were identified by biochemical tests. They were identified as *Bacillus* isolates and were also identified using Vitek 2. The technique was able to diagnose only 7 isolates. The isolates were then tested to produce antimicrobial agents against 5 known bacterial pathogens *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonasaeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*. Most isolates showed a positive result against these pathogenic bacteria and then isolated 9 isolates using 16SrRNA gene for PCR products. All isolates were positive for this gene. After treatment for each isolate using Mega 6 program, and obtained new isolate and get code MF423696 after matching them with NCBI. And 5 isolates were selected for the purpose of completing the study. The antibiotic was then extraction by centrifugation at 10.000 cycles / minute for 15 minutes and then filtered using 0.22µm micrometer filter. It was used to inhibit the pathogenic bacteria. A set of specific reagents was carried out to identify the active substances found in the extract including detection of phenols, flavonoids, amines, and saponins**.** All isolates were positive for phenols, flavonoids, amines, and saponins except RL2 that showed negative to this test.

Keywords : *Bacillus* sp., 16SrRNA, Antibiotics.

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الخلاصة

تهدف الدراسة الحالية إلى عزل و تشخيص عزلات *Bacillus* spp. محلية منتجة للمضادات الحياتية من بعض ترب محافظة ذي قار و الكشف عن وجود جين 16SrRNA بإستعمال تقنية DNA Sequence إذ تضمنت الدراسة جمع 25عينة تربة من مناطق مختلفة من محافظة ذي قار و هي الرفاعي و النصر و الشطرة و الغراف و الناصرية و تم عزل 13 عزلة بكتيرية اعتمادا على اشكال و الوان المستعمرات بالإضافة إلى عزلتين مشخصتين جينيا مسبقا هما RL1 و RL2. شخصت البكتريا باستعمال الاختبارات الكيموحيوية و جهاز الفايتكإذ تمكنت هذه التقنية من تشخيص 7عزلات فقط . اختبرتجميع العزلات لمعرفة قدرتها على إنتاج المواد ضد ميكروبية بأخذ أقراص من العزلات ووضعها على وسط Mueller- Hinton المزروع بأحد أنواع البكتريا المرضية و التي تضمنت *K. pneumonia* و *P. aeruginosa* و *P. mirabilis* و *Salmonella typhi* و *Staphylococcus aureus* و المشخصة باستعمالمجموعة من الإخباراتالكيموحيويةو نظام API20 E و API20 Strip إذ أعطت بعض العزلات نتيجة موجبة و بعضها سالبة. شخصت 9 عينات باستعمال جين 16SrRNA و كانت جميع العينات نتيجة موجبة لهذا الجين و تم تسجيل عزلة بكتيرية جديدة في البنك الجيني و اعطيت الكود MF423696 و تم اختيار 5عزلات منتجة للمضادات الحيوية لغرض إجراء الدراسة. استخلص المضاد الحياتي بنبذ وسط الإنتاج في جهاز الطرد المركزي بسرعة 10.000 دورة/ دقيقة لمدة 15 دقيقة ثم رشح المستخلص بواسطة اوراق ترشيح بحجم 0.22 مايكروليتر و استعمل المستخلص لمعرفة فعاليته التثبيطية ضد البكتريا المرضية المختارة للدراسة. استعملت مجموعة من الكواشف للكشف عن المواد الفعالة الموجودة في المستخلص من أهمها الكشف عن الفينولاتوالفلافينويدات و الامينات والصابونيات

Introduction

Bacillus species are gram positive bacteria, road shaped, spore forming and possesses many physiological abilities that allow it to live in multiple environments (Al-Allaf, 2011). Soil is the main habitat for species of this bacteria that found on plant roots and also with rhizosphere or aerial parts. It has a role in helping plants to obtain nutrients, however protecting them from disease and helping them to produce plant hormones. This bacteria is the most abundant soil genus available in the root soil area (Lyngwi and Joshi, 2014). Under stressful environmental conditions, the bacteria can produce oval endospores that are not true spores but which the bacteria can reduce themselves to and remain in a dormant state for very long periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera of Firmicutes (Torome, 2015). Some Microorganisms are able to synthesize secondary metabolites of various structures and, hence, bioactivities. Their production is regulated by nutrients, growth rate, enzyme inactivation and induction ,they are produced to help the organism in competing successfully with other organisms in their natural habitat and to adapt with the changed environmental conditions(Al-Sarairehet *al.,* 2015).

Antibiotics are known as low molecular weight complex chemicals, secondary metabolites produced by microorganisms that are effective against other microorganisms(Singh and Meshra, 2013).Several studies have confirmed the ability of some *Bacillus* species to produce effective antimicrobial compounds against bacteria and fungi (Baruzzi*et al.,* 2011). The number of antibiotics produced by these species is 167, of which 66 are produced by *Bacillus subtilis* and 23 are by *Bacillus breives* and the remainder are produced by other species (Awais*et al.,* 2010).

Antibiotics can be classified according to their mode of actions as broad-spectrumantibiotics when they have the ability to affect a wide range of gram-positive and gram-negative bacteria whileantibiotics that only effective towards certain group of bacteria are known as narrow-spectrum antibiotics (Lihan et *al.,* 2014). The method of antibiotic effect on the cells depends on several mechanisms, including antibiotics, which inhibit the manufacture of the cell wall of bacteria and antiviral agents to prevent DNA replication and repair by influencing the enzymes of gyrase, topoisomerase and N methyltransferase, and other inhibit the process of protein synthesis by inhibiting the work of the units of ribosomal and other Interferes with the metabolic processes in the cell by influencing the enzymes responsible for the manufacture of many important compounds of the cell (Usha, 2011). Antibiotics produced by this genus can be classified by their genetically modified peptide antibiotics, known as bacteriocyanates, enzymatically induced peptide antibiotics and non-peptide antibiotics (Torome, 2015). Because of the increasing numbers of antibiotic resistant

bacteria, there is a need to look for new antibiotics by looking for more effective ways to isolate bacteria and fungus (Nordenfjall, 2014).

The aims of this study was to isolate and diagnosis of local bacterial isolates that produced antimicrobials from soil samples, detecting the presence of 16SrRNA gene by using DNA sequence technique and test the ability of bacteria to produce antibiotics against some species of pathogenic bacteria.

Materials and Methods

Sample collection

Twenty-five soil samples were collected from different areas of the Thiqargovernorate after removal the surface layer of the soil. The sampleswere taken from a depth of 3-15 cm. Each gram of sample was suspended in 9 ml of sterile distilled water and shaken vigorously for 2 minutes. The soil suspension were serially diluted in sterile distilled water by take 1 ml of soil suspension and put in 9 ml of sterile distilled water and so to the sixth dilution and the dilutions from 10^{-1} to 10^{-6} were heated at 60˚C for 60 minutes in water bath (Aslim andBeyatli, 2002). 1 ml of each dilution was poured and spreader over nutrient agar plates by using sterile Lrod and incubated at 37 ° C for 24 hours for isolation.

Diagnosis

Microscopic Diagnosis

Gram staining was used to determine the nature of the bacterium and used Malachite green stain to staining the endospores.

Biochemical Reactions

A variety of tests were used for the purpose of diagnosing these bacteria, including starch hydrolysis, Simone citrate fermentation test, mannitol fermentation, oxidase test, catalase test, blood hydrolysis, Methyl red test,Voges-Proskauer test, Motility test, Gelatin liquefaction test.

Diagnosis by Vitek 2 system

These bacteria were identified by this technique using BCL cards that containing 46 tests.

Molecular study

The genetic material was extracted by using the boiling modified method as follows:

1- Growth the bacteria on nutrient agar mediumand incubated at 37 ° C for 24 hours.

2- Place 50 microliters of ddH2O in an eppendorf tube of 1.5 ml.

3-Transfer the twice to three times from the bacterial growth to the Eppendorf tube and mix well,

4- The isolates were placed in the water bath for 5 minutes at 100 ° C.

5- Centrifugation the mixture with a refrigerated centrifuge at 5000 rpm for 5 minutes.

6- Mixed the precipitate with the floating part by vortex.

7- DNA was detected by electrophoresis at 1% concentration for 1 hour and 100 volts.

16SrRNA gene

The presence of this gene was detected using the 1942 forward primer and 27 reverse primer (Pradhap*et al.*, 2011)

1- Add 5 microliters of DNA to the Master Mix tube.

2- Add 1 microliter of each forward and reverse primer.

3- Add 8 microliters of deleted distilled water to make the desired final size 20 microliters.

4- Mix ingredients together with Centrifuge.

5- The tubes are placed in a Polymerase chain reactionthermal Device.

Test of the ability of bacteria isolated on the production of antibiotics

Testing of the ability of bacteria isolated to production of antibiotics by using Kirby power method (Atsede, 2011) as following:

1- The isolates were growth on nutrient agar medium and incubated at 37 ° C for 7 days.

2- After growth, 7 mm tablets were removed from the colony of the bacteria by thorny Fellini sterilized. The disks were then placed on the Mueller Hinton mediumthat containing with pathogenic bacteria.

3- Place the dishes in the refrigerator at a temperature of 4 C for 18 hours to ensure the spread of materials against microbial and incubate the dishes at 37 ° C for 24 hours.

4- Detection of the efficacy of isolates against pathogenic bacteria by the growth of isolates around the disks.

Antibiotic extraction

The development of the isolates was carried out in the center of the nutrient broth for 24 hours in the shaking incubator in 150 rpm and 37 C˚ and then grown in the production mediumthat contain Glucose 1%, Peptone 1%, Malt extract 0.5%, NaCl 0.3%, Glycerin 0.25% (Shoji et al., 1977).

Centrifugation the medium in the cooled centrifuge device at 1000 rpm for 15 minutes and then filter using a filter paper 0.22 microliters and keep at 4 ° C until use.

Test Bacteria

Staphylococcus aureus, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Salmonella typhi* were used as test bacteria and were get it from laboratory of graduate studies ,Biology department, college education of pure science, University of Thiqar.

Sensitivity to the Antibiotics Extraction

This operation was done depending on (Torome, 2015) as following:

1- *S. aureus* was grown on the blood base agar medium while the remaining isolates were grown on a nutrient medium at 37 ° C for 24 h.

2- A bacterial suspension was performed by placing 5 ml of the phosphate salt solution in a test tube and added one colony of pathogenic bacteria and reduced the turbidity by comparing with 0.5 standard McFarland tube.

3- Squeeze a sterilized soap into the tube and press the tube walls to get rid of the liquid.

4- Kirby power method was used to spread the bacteria on the Mueller Hinton medium and leave the dishes for 10 minutes to dry.

5-Used a 7 mm diameter thorny Fellini to make drills in the medium and put the 200 microliter in the drill. After that, left the plates in the refrigerator for 18 hours and incubated at 37 C° for 24 hours.

Sensitivity to the Antibiotic Disks

Testing the pathogenic bacteria to variety of antibiotics include Amikacin, Gentamicin, Imepinime, Tetracycline, Trimethoprim, Tobramycin, Vancomycinby growing pathogenic bacteria on nutrient agar medium and put antibiotics disks on this medium and incubated at 37 C° at 24 hours.

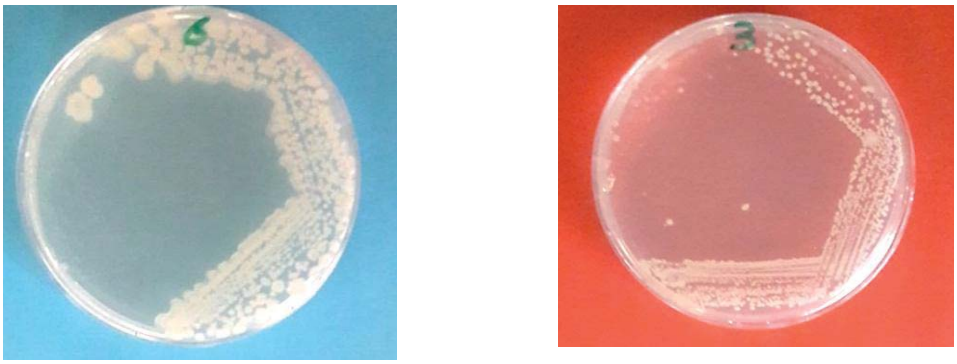
statistical analysis

Statistical comparisons of the resultswere performed by one-way ANOVA using SPSS ver. 19.Significant differences ($P<0.05$) among bacterial pathogens were analysed by Duncan triplicates range test.

Results

Isolation

In this study was collected 25 soil samples and plated on nutrient agar plates and 13 isolates were isolated and diagnosis morphologyon the basis of colony color, strength and shape as shown in Fig1



A- The phenotypic form of isolation *B. anthrax* EFF-M15 on the Nutrient Agar medium in the lap period 37 ˚C

B-phenotypic form of *B. thuringiensis* Bac-thnonoor isolation on the Nutrient Agar medium in the lap period 37 ˚C

Figure (1): The phenotypic diagnosis of isolated bacteria

All isolates were positive for Gram stain and containing endospores afterstaining with malachite green stain as shown in Fig (2,3):

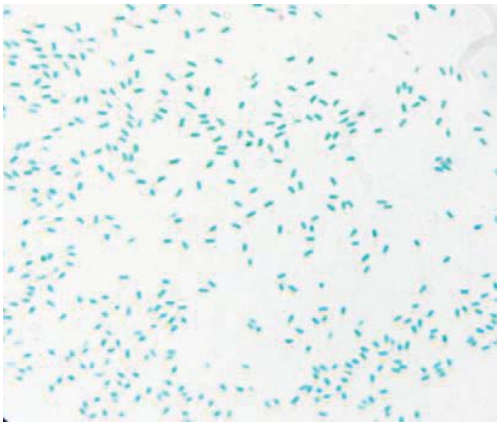


Figure (2) Detection of internal spores

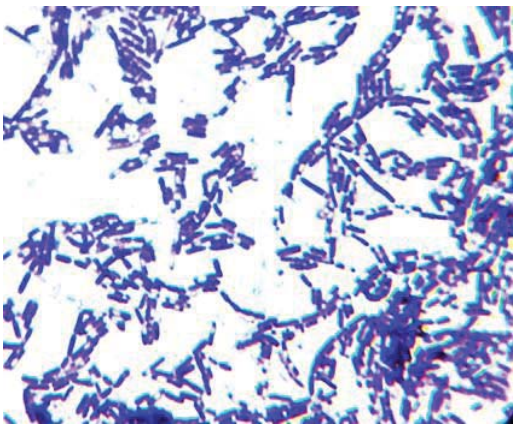


Figure (3): Gram Stain

Biochemical Reactions

This bacteria was identification by some of the biochemical tests such as Gram stain, Endospore stain, starch hydrolysis, citrate utilization, blood hydrolysis, mannitol, lactose, glucose fermentation, methyl red, voges-proskauer, indole, gelatin liquefaction, motility, catalase, oxidase, lecithenase test as shown in Table (1)

Table (1): Biochemical tests used in diagnosis of bacteria isolated

Test	B 1	B 2	B3	B4	B5	B 6	B7	B8	B9	B10	B11	B12	RL 1	RL 2	B15
Gram stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endospore stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	-	+	-	+	+	+	+	-	-	+	+	-
Blood hydrolysis	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Mannitol fermentatio	+	+	+	+	-	+	+	-	-	-	-	-	+	+	+

n															
Lactose fermentation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Glucose fermentation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl Red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	+	+	-	+	-	-	+	-	-	+	+	+	+	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	+	-	-	+	-	-	-	-	-	-	-	+	+	+	+
Motility	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	+	-	+	-	-	-	+	+	+
Lecithenase	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-

(+): positive , (-): negative

Diagnosis by Vitek 2 System

The isolates were identified by this device using the BCL Cards Kit and the results were as shown in the table (2)

Table (2): Diagnosis of bacteria by Vitek 2 system

Isolate	Identification	Identification %
B1	*Bacillus cereus*	**87 %**
B2	*Bacillus cereus* *Bacillus thuringiensis* *Bacillus mycoides*	**90 %**

B3	*Bacillus cereus* *Bacillus thuringiensis* *Bacillus mycoides*	**93 %**
B4	*Bacillus pumilus*	**87 %**
B5	Unidentified organism	
B6	*Bacillus subtilis* *Bacillus amyloliquefaciens* *Bacillus atrophaeus*	**93 %**
B7	Unidentified organism	
B8	Unidentified organism	
B9	*Bacillus cereus* *Bacillus thuringiensis* *Bacillus mycoides*	**87 %**
B12	*Bacillus cereus* *Bacillus mycoides*	**89 %**
RL1	Unidentified organism	
RL2	Unidentified organism	
B15	Unidentified organism	**99 %**

Molecular study

DNA was detected by the migration of the genetic material on 1 % agarose as show in figure (4):

Nine samples were sent after confirming the existence of the diagnostic 16SrRNA gene to the Korean company Macro gene for diagnosis using 16SrRNA PCR sequence. A new isolate was obtained. B3 was diagnosed as *Bacillus thuringiensis* , after treatment with Mega 6 program, a new strain was obtained and get the code MF423696 in NCBI. B6 was also identified as *Bacillus anthracis* .

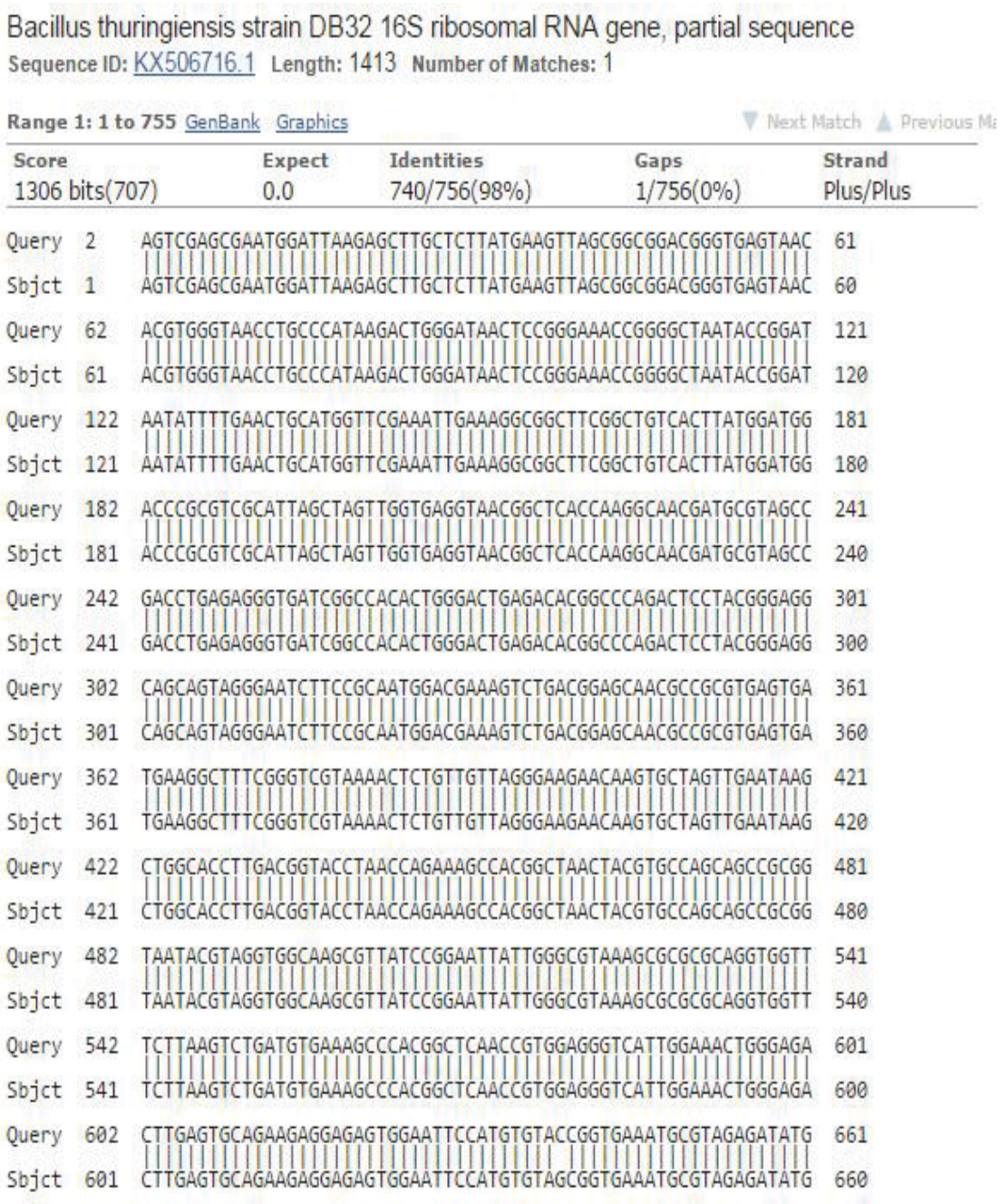


Figure (6): BLAST of *Bacillus thuringiensis* Bac-th/noor-1

Bacillus anthracis strain EFF-M15 16S ribosomal RNA gene, partial sequence

Sequence ID: KP813662.1 Length: 1241 Number of Matches: 1

Range 1: 1 to 1241 GenBank Graphics Next Match Prev

Score	Expect	Identities	Gaps	Strand
2292 bits(1241)	0.0	1241/1241(100%)	0/1241(0%)	Plus/Plus

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Query  1    TGGATGACGCATGGTCACTGCAGTCGTGCGAATGTGATCTCAGAGAGCTTGCTCTTATGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGGATGACGCATGGTCACTGCAGTCGTGCGAATGTGATCTCAGAGAGCTTGCTCTTATGA  60

Query  61   AGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTC  120

Query  121  CGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGC  180
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Sbjct  121  CGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGC  180

Query  181  GGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGG  240

Query  241  CTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAG  300
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Query  301  ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC  360
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Sbjct  301  ACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC  360

Query  361  TGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGG  420
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Sbjct  361  TGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGG  420

Query  421  GAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACG  480
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Sbjct  421  GAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACG  480

Query  481  GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATT  540
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Query  601  TGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTG  660
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Sbjct  601  TGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTG  660
```

Figure (7): BLAST of *Bacillus anthracis* EFF-M15

Test the ability of bacterial isolates to produce antibiotics

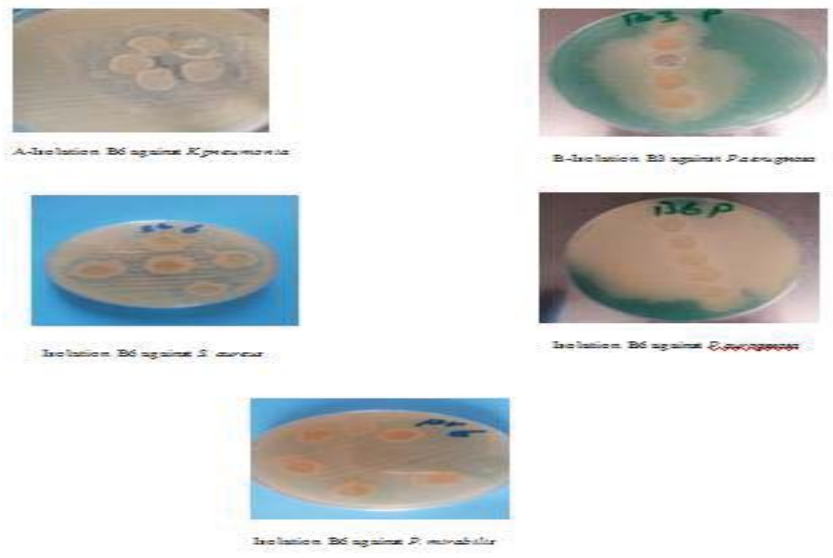
Bacterial isolates were tested for their ability to produce antibiotics against five types of pathogens previously mentioned. The results were as shown in Table 3:

Table (3): Ability of bacteria to produce antibiotics against pathogenic bacteria

Isolate	*P. aeruginosa*	*S. typhi*	*K.* pneumoniae	*S. aureus*	*P. mirabilis*
B1	+	-	-	+	-
B2	-	-	-	+	-
B3	+	-	-	+	-
B4	+	-	-	+	-
B5	-	-	-	-	-
B6	+	+	+	+	+
B7	+	+	+	+	+
B8	+	+	+	-	-
B9	+	-	-	-	-
B10	-	-	-	-	-
B11	-	+	-	-	-
B12	-	-	-	-	-
RL1	-	-	-	-	-
RL2	+	+	+	+	+
B15	+	+	+	+	+

(+): positive (-): negative

After test ability of bacteria to produce antimicrobials were results as shown in figure (8)

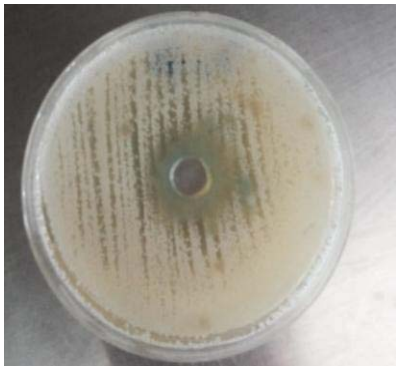


Figure(8): Ability of bacteria to produce antibiotics

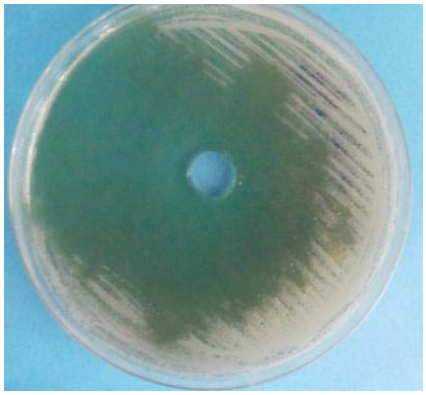
Antibiotic	Pathogenic Bacteria				
	S. typhi	*K. pneumonia*	*P. mirabilis*	*S. aureus*	***P. aeruginosa***
B.thuringiensis Bac-th/noor-1	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{d}	**0.00^{b}**
B. pumilus	0.00^{d}	0.00^{b}	0.00^{b}	0.00^{c}	**0.00^{b}**
B. anthracis EFF-M15	40.00^{a}	0.00^{b}	0.00^{b}	0.00^{d}	**0.00^{b}**
B. subtilis DP10	20.00^{c}	0.00^{b}	0.00^{b}	20.00^{c}	**0.00^{b}**
Bacillus sp. RL2	30.00^{b}	20.00^{a}	20.00^{a}	70.00^{a}	**30.00^{a}**

Sensitivity to the Antibiotics Extraction

The sensitivity of the pathogenic bacteria to the antimicrobial agents was tested and the result was as shown in the table (4):



A- The inhibitory ability of isolation B15 against *S.aureus*



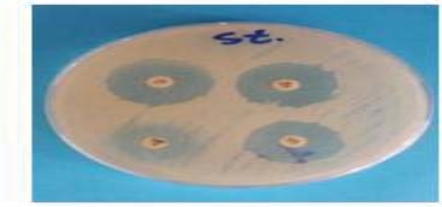
B-The inhibitory ability of RL2 isolation against *S. aureus*

Table (9): Shows the most important results of the sensitivity test of bacterial pathogens

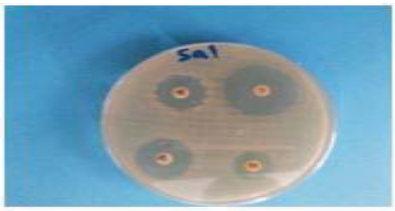
The sensitivity of the bacteria to seven antibiotics was tested to compare them with the bacterial sensitivity of the antibiotic extracts and the results were close

Table (5): The sensitivity test of pathogenic bacteria to extract antibiotics

Antibiotic	**Bacteria**				
	S. typhi	*K. pneumonia*	*P. mirabilis*	*S. ayreus*	***P. aeruginosa***
Amikacin	10.00^{b}	10.00^{b}	2.00^{b}	20.00^{c}	**2.00^{a}**
Gentamycin	10.00^{b}	10.00^{b}	2.00^{b}	20.00^{c}	**2.00^{a}**
Imipenem	20.00^{a}	20.00^{a}	2.00^{b}	40.00^{a}	**2.00^{a}**
Tetracycline	10.00^{b}	20.00^{a}	10.00^{a}	30.00^{b}	**2.00^{a}**
Tobramycin	10.00^{b}	10.00^{b}	10.00^{a}	20.00^{c}	**0.00^{b}**
Trimethoprim	20.00^{a}	0.00^{c}	2.00^{b}	10.00^{d}	**2.00^{a}**
Vancomycin	0.00^{c}	0.00^{c}	2.00^{b}	20.00^{c}	**2.00^{a}**

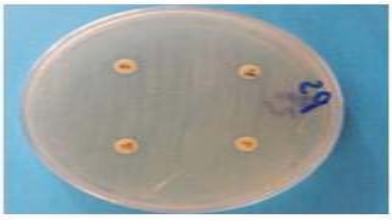


A-Sensitivity test of *Staphylococcus aueusto* Tobramycin, Amikacin, Trimethoprim and Vancomycin



B-Sensitivity test of *Salmonella typhito* Tobramycin, Trimethoprim and Imipenem

C-Sensitivity testing of *Proteus mirabilisto* Tobramycin, Tetracycline, Imipenem and Gentamycin



D-Sensitivity test of *Pseudomonas aeruginosa*Tobramycin, Amikacin, Trimethoprim and Vancomycin

Discussion

Bacteria is one of the most important components of the soil, as the sowing of a sample of soil on the center of nutritious and then exposed to heat to kill the green cells and the growth of the remaining whiteboards, which leads to easy to study taxonomic and physiological and these bacteria can withstand high temperatures and have the ability to secretion the extracellular enzymes are commercially important and are also important in biological treatment through their ability to analyze BTEX compounds (Benzene, Toluene, Ethyl benzene and Xylene) and produce a wide range of antiviral, bacteriological and fungal compounds that play an important role in microorganisms interactions with some of them inside the soil and these compounds of medical and agricultural importance.

The results of the present study showed that all isolated isolates were positive for Gram stain and this was obtained by Kumar *et al.*,(2012). The isolates were identified by a combination of biochemical tests including starch analysis. All isolates were positive for this test and blood decomposition, mannitol fermentation, sugars fermentation, methyl read test, voges-prpskauer test, indole test, gelatin hydrolysis, motility test, catalase, oxides and lecithenase production..

Eleven isolates were diagnosed by Vitek 2 and BCL cards were used to diagnose only 7 isolated isolates. The remaining 4 isolates were not diagnosed and the diagnosis ranged between 87-93%. This device was used in diagnosis for its ability to give the result within a period of short and accurately and is developed by Bio merieux company and does not need to add any material except processed by the company and its attached either way it works automatically without having to enter working on it and gives the results in an easy way as to be linked to a calculator electronic is The results can be easily obtained by the researcher It is about 99%.

The bacteria were tested for their ability to produce antibiotics by Atsede (2011) method. The result was to obtain some isolates capable of secreting these substances such as B1, B3, B4, B6, B7, RL2 and B15 (Abo-Shadi*et al.*, 2010). All microorganisms isolated from soil are a good source of secondary antimicrobial agents. Metabolic substances that produce by these genus are characterized by its protein nature and synthesized by ribosomes in the cell bacterial that have different structures and molecular weights and mechanisms against pathogen bacteria (Muhammad, 2015).

Nine samples were identified using 16SrRNA. New isolates were obtained after treatment and compared to NCBI and recorded in the gene bank. Current results showed that all isolates were positive for the 16SrRNA gene that located at 1500 base pair.

In addition to the antibiotic test and the extract, *P. aeruginosa* was resistant to the selected antibiotics and to the bacterial extract except Rl2, which gave inhibitory power against these bacteria.

Salmonellatyphi was sensitive to the antibiotics of Imipenem and Trimethoprim and resistant to the rest of the antibiotics while they were sensitive to isolates B6 , B15 and RL2 .*Klebsiella pneumonia*was sensitive to Imipenem, Tetracycline and RL2 isolates. *Staphylococcus aureus* was sensitive to all antimicrobials except Trimethoprim while resistant to all isolates except RL2 and *Proteus mirabilis* was resistant to all antibiotics while sensitive to RL2 isolate.

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