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Isolation and identification of Rhizobium bacteria from Faba bean (*Vicia faba L.*) roots grown in gypsiferous soil and testing their efficiency in production growth regulate(IAA) and siderophorses

ABSTRACT

In this study 30 samples were collected from the root nodules of Faba bean plants (Vicia faba L.) grown in gypsiferous soil in the College of Agriculture / Tikrit University / Salah al-Din Governorate in 5/12/2020, and the isolation process was carried out on selective mediumYeast Extract Mannitol Agar , and the phenotypically pure isolates were identified based on the culture, microscopic and biochemical characteristics, and the phenotypic identification results showed that 23 isolate gave the characteristics of Rhizobium sp. ,it was color of the colonies between white, cream and yellow, shiny, convex and smooth, gram-negative and most of able to move and has color between light pink and white on Congo red stain medium (0.025%) and it most of fast growing giving them yellow color on bromothymol blue medium and unable to grow on Hofer alkaline medium, and the efficient isolate was selected in the production of indole acetic acid, chelating compounds, and identificated molecularly by PCR, the 16SrRNA gene was amplified, then the sequence of nitrogenous bases, the results showed that there is a 99.69% similarity with the Rhizobium leguminosarum bv. Viciae strain SMV12a, and therefore the isolate is genetically close to Rhizobium bacteria.

INTRODUCTION

Rhizobium bacteria is a genera of aerobic bacteria belonging to the phylum Proteobacteria, the class Alphaproteobacteria, the order Rhizobiales, the family Rhizobiaceae, and the genus Rhizobium, and located under several bacterial genera the class Alphaproteobacteria, namely *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Allorhizobium*, *Mesorhizobium*, *Agrobacterium*, *Phyllobacterium*, *Ochrobacterium*, *Methylobacterium*, *Shinella*, and under the class Betaproteobacteria, other genera are *Burkholderia*, *Cupriavidus* and *Herbaspirillum* (Pervin et al., 2017 ;Lindström et al, 2010), It is a gram-negative bacteria, rod-shaped with a length of 1.2-3 µm and a width of 0.5-0.9 µm, It is aerobic, non-spore-forming, motile by peritrichous or polar flagella, and it is present in the soil freely or lives symbiotically within the tissues of the plant, and grow these bacteria in the form of white or creamy colonies and mucous or semi-mucosal and convex on

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medium containing on Yeast Extract Mannitol Agar(YEMA) which is the special medium for isolating and purifying root nodule bacteria,and consume these bacteria carbohydrates as a source of carbon, ammonia, urea and nitrate as a source of nitrogen, and have the ability to withstand different effects of temperature and pH, as the optimum temperature for its growth is 28-31°C and pH 6-7 (Vincent,1970; Ahmed et al ,2019), and from of the necessary characteristics in identification root nodule bacteria is its ability to infect leguminous plants and speed of their growth on YEMA medium, which are classified into two main groups depending on the generation time, and they are fast-growing species such as *R.leguminosarum* that infects Faba bean, pea and lentil plants,and *R.trifoli* and *R.meliloti* they infect alfalfa and medicago plants, respectively, and *R. phaseoli*, which infects common bean plants, and slow-growing species such as *Bradyrhizobium* sp. that infects cowpea, peanut, lablab and soybean, and on this depends the success of root nodules bacteria in fixing nitrogen when it is associated with the leguminous plant (Zahran,2001),confirmed Setargie et al. (2015) that the eight *Rhizobium* isolates taken from the soil surrounding the roots of the lentil plant *(Lens culnaris)* they were identificated based on agronomic, biochemical and physiological characteristics, and they showed rod shapes and different colors ranging from white to yellow, sticky texture, gram-negative, rod-shaped, and no its ability to absorb Congo red stain when grown on YEMA-C.R medium and gave positive results for catalase and H_2S production tests and negative results for urease and citrate tests. As for the growth ability of bacterial isolates at different pH levels from level to another, as all isolates showed good growth at the pH level (7-8) and not growing at level (4), and this indicates that the strains of *R.leguminosarum bv.vicia* are sensitive to low pH and grow well at pH neutral,and identified Al-Samarrai (2017) Rhizobia bacteria isolated from different leguminous plants, including cowpea, soybean and mung bean, depending on the agronomic, microscopic and biochemical characteristics, and he pointed that all bacterial isolates after growing on the special YEMA medium were spherical, convex, smooth, mucous, motile, and gram negative, and most of they were fast-growing on the medium, with colors ranging from white, yellow, and cream, and their inability to absorb the Congo red stain, most of the isolates were able to change the color of the medium containing bromothymol blue stain from green to yellow because of their ability to produce compounds that increase the acidity of the medium due to the fast-growing groups, and positive for the keto-lactose test, and that most of the isolates do not have the ability to grow on alkaline Hofer medium and have the ability to grow on solid glucose-peptone medium because they consume glucose sugar as a source of carbon, and positive tests for catalase, citrate, triple sugar, iron and lactose and these tests are the most important characteristics of the root nodule bacteria.

MATERIALS AND METHODS

A laboratory experiment was conducted to isolate and identification of *Rhizobium* bacteria taken from root nodules of Faba bean plants grown in gypsiferous soil containing 90.6 gm of gypsum kg^{-1} soil.

Isolation of *Rhizobium* bacteria from the root nodules

The roots were washed and the pink active root nodules were selected and then sterilized using ethyl at a concentration of 95% for 5-10 seconds and then washed with sterile water 3-4 times, then immersed the root nodules in $HgCl_2$ concentration of 0.1% for 3-4 minutes, then washed with sterile water at least 6 times (Somasegaran and Hoben ,1994), Then the root nodules were transferred into new dishes and crushed using a sterile glass rod under sterile conditions to obtain a bacterial suspension, then was taken 1 ml of the bacterial suspension using a sterile pipette and cultured on YEMA medium poured into sterile plastic dishes and the dishes were incubated at temperature 28 ˚C for 3-7 days then purification of the bacterial isolates (Vincent, 1970).

Identification of *Rhizobium* bacteria

The process of identification bacterial isolates was carried out on the basis of several culture characteristics such as: shape,texture,color,convexity and growth, and microscopically using Gram stain and motility test by stabbing method (Collee et al, 1996).

1-Congo red stain test

The test was carried out to differentiate between root nodules bacteria and *Agrobacterium* by absorbing the stain, as the bacteria were cultured and incubated at a temperature of 28°C for 3-5 days, The results were recorded on the basis of their ability to absorb the stain, as the root nodule bacteria do not absorb and remains white, while the other genera are colored red (Pervin et al, 2017; Vincent, 1970).

Table (1): component of Congo red test

Material	Weight (gm L^{-1})
Mannitol	10.00
$MgSO_4.7H_2O$	0.200
NaCl	0.100
K_2HPO_4	0.500
$CaCl_2.2H_2O$	0.200
$FeCl_3.6H_2O$	0.0100
Yeast extract	1.00
Agar	20.00
Congo red	0.025

2-Bromothymol blue stain test

This test is used to identify the bacterial genus of the slow or fast-growing groups *Rhizobium* or *Bradyrhizobium* by the ability of the bacteria to produce acid or base resulting from a change in the color of the medium containing the stain from green to yellow or blue, as the medium was prepared then inoculated with the isolated bacteria and incubated at a temperature of 28°C for 2-7 days, then the results were recorded (Somasegaran and Hoben,1994).

Table (2): component of Bromothymol blue test

Material	Weight (gm L^{-1})
Mannitol	10.00
$MgSO_4.7H_2O$	0.200
NaCl	0.100
K_2HPO_4	0.500
$CaCl_2.2H_2O$	0.200
$FeCl_3.6H_2O$	0.0100
Yeast extract	1.00
Agar	20.00
Bromothymol blue	0.500

3-Hofer alkaline test

This test is a way to distinguish between root nodules bacteria and *Agrobacterium* because the genus *Agrobacterium* has the ability to grow at high levels of pH (pH 11), while root nodules bacteria do not grow at these levels, as the medium was inoculated with a pure smear of the colonies were incubated at 28°C for 48 hours(Pervin et al.,2017) .

Table (3): component of Hofer alkaline test

Material	Weight (gm L^{-1})
Mannitol	10.00
$MgSO_4.7H_2O$	0.200
NaCl	0.100
K_2HPO_4	0.500
$CaCl_2.2H_2O$	0.200
$FeCl_3.6H_2O$	0.0100
Yeast extract	1.00
Agar	20.00
Thymol blue	0.016

4- Keto-lactose test

The dishes containing the test medium were incubated after inoculation with pure colonies for 3-5 days, and after the incubation period ended, the dishes were covered with a small layer of Benedict's reagent and then incubated for an hour to determine the purity of bacteria by the absence of the yellow areas around the colonies, while the appearance of the yellow color around them indicates the presence of other genera.

Table (4): component of Keto-lactose test

Material	Weight (gm L^{-1})
Lactose	10.00
$MgSO_4.7H_2O$	0.200
NaCl	0.100
K_2HPO_4	0.500
$CaCl_2.2H_2O$	0.200
$FeCl_3.6H_2O$	0.0100
Yeast extract	1.00
Agar	20.00

5- Tests for oxidase, catalase, citrate, urea, gelatin and triple sugar iron

These tests were conducted according by (Collee et al ,1996).

6- Efficiency of isolates to produce indole acetic acid

The test was carried out according to the method of (Patten and Glick ,2002), It was prepared of the peptone water by solubilizing 10 g of peptone in 990 ml of distilled water, then completed the volume to a liter and distributed in test tubes of 10 ml for each tube, then inoculated and incubated at 28 ± 2°C for 24 hours, and then the indole produced in the medium was estimated using the colorimetric method, by taking 1 ml of the culture after centrifuging it at a speed of 3000 rpm^{-1} for 15 minutes, then adding to it 1 ml of the previously prepared Salkowsky reagent and leaving it in the dark for 30 minutes at 25-30°C, the absorbance was measured with a spectrophotometer at a wavelength of 535 nm.

7- Testing the efficiency of isolates in producing chelating compounds

The test was conducted based on the method described by (Payne,1980), where a liter of solid nutrient medium was prepared and the pH was adjusted to 7.2, sterilized by oxidation and left to cool to a temperature of 46-48 °C, and then 2 mg of 2.2'-dipyridyl solubilized in 10 milliliters of sterile distilled water by 0.2 µm diameter microfilters to obtain a final concentration of 200 µg ml^{-1}, and incubated at 28°C for 24 hours, the appearance of growth is evidence of the bacteria's ability to produce iron chelating compounds.

8- Molecular identification

Rhizobium bacteria were molecularly identified using (Gradient PCR) technique by extracting and purifying the DNA of root nodules bacteria and detecting the presence of bacterial DNA using the primer shown in the table below:

1.Polymerase Chain Reaction (PCR)

1- Maxime PCR PreMix kit (i-Taq) 20µlrxn (Cat. No. 25025).

2- The 16SrRNA gene was duplicated using PCR technique with a pair of primers, and the universal primer whose sequence is shown in Table 5 to determine the selected isolates of the ITS region (Miller et al., 2013) prepared from (Integrated DNA Technologies company, Canada).

Table (5): The primer used in the molecular identification of root nodules bacteria

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair
Reverse	5'- GGTTACCTTGTTACGACTT- 3’	49.4	42.1	

2.Acarose gel electrophoresis

Thermal polymerization device was used (Applied Bio-system Gene-amp PCR System 9700) in the process of duplication of the mentioned gene, Table 6 included the standard reaction conditions, and the separation of the PCR product using electrophoresis on agarose gel (1.5%), then showed the genetic DNA bundles using UV rays At a wavelength of (302nm) after treatment with the stain (Intron Korea red stain).

Table (6): polymerase chain reaction conditions

No.	Phase	Tm(C)	Time	No.of cycle
1	Initial Denaturation	95 ˚C	5min	1 cycle
2	Denaturation	95 ˚C	45sec	35 cycle
3	Annealing	58 ˚C	45sec	
4	Extension -1	72 ˚C	45sec	
5	Extension -2	72 ˚C	7min	1 Cycle

3.Sequencing Analysis:

The nucleotides of the PCR amplified gene were sequenced immediately after obtaining the 16SrRNA gene duplication by sending a volume of 25 µl of PCR product and a volume of 10 µl (10 pcm) of each primer to the Korean company Biotechnology Lab (used device Applied Bio-system 3730XL, DNA Sequencer),The results were compared through a computer program connected to the web (Basic Local Alignment Search Tool (BLAST) with the database of the National Center for Biotechnology Information (NCBI) in which the matching of the nucleotide sequences of the gene is performed). 16SrRNA for bacterial isolates were included in the research and their type was determined according to the conformity in the aforementioned database. After the diagnosis of bacterial isolates was completed, the percentage of similarity with the global strains recorded in the genetic bank and the global number for each strain was recorded.

RESULTS AND DISCUSSION

1-Cultural and microscopic characteristics

Table 7 shows the twenty-three isolates belonging to the family Rhizobiaceae isolated from the root nodules of Faba bean plants growing in gypsiferous soil, which were identificated based on the cultured and microscopic characteristics, and the table shows that 16 isolates grew after being incubated for 3-5 days and at a temperature of 28°C, which was classified as belonging to the fast-growing *Rhizobium* bacteria, while 7 isolates grew on the same medium and belonging to the slow-growing *Bradyrhizobium* bacteria, This result agrees with the results of Al-Samarrai (2017) in his study of root nodule bacteria isolated from different leguminous plants including soybean, mungbean, and cowpea, all of the study isolates were characterized by being convex, smooth and mucous, while most of the colonies were rod-shaped, with the except of 5 isolates (R2, R3, R5, R6, R17), which showed spherical shapes and colors from white, cream and yellow, As for the microscopic characteristics shown in table 7 all isolates showed that they are gram-negative and most of them are capable of motility, as this result converges with the results of Elzanaty et al. (2015) in their study on *Rhizobium* bacteria isolated from the root nodules of Faba bean plants from different regions of Egypt and they found that the isolates are gram-negative and it has the ability to move.

Table (7): Cultural and microscopic characteristics of Rhizobium bacteria isolated from root nodules of Faba bean plants

isolates	growth	shape	convexity	mucus	texture	Gram	color	Movement
R1	fast	rod	convex	mucous	smooth	-	White	Motile
R2	fast	spherical	convex	mucous	smooth	-	Yellow	Motile
R3	fast	spherical	convex	mucous	smooth	-	white	Motile
R4	fast	rod	convex	mucous	smooth	-	white	Motile
R5	fast	spherical	convex	mucous	smooth	-	white	Not motile
R6	fast	spherical	convex	mucous	smooth	-	white	Motile
R7	slow	rod	convex	mucous	smooth	-	white	Motile
R8	fast	rod	convex	mucous	smooth	-	white	Not motile
R9	fast	rod	convex	mucous	smooth	-	white	Motile
R10	fast	rod	convex	mucous	smooth	-	white	Motile
R11	fast	rod	convex	mucous	smooth	-	white	Motile
R12	fast	rod	convex	mucous	smooth	-	white	Motile
R13	fast	rod	convex	mucous	smooth	-	creamy	Not motile
R14	fast	rod	convex	mucous	smooth	-	creamy	Motile
R15	fast	rod	convex	mucous	smooth	-	Creamy	Motile
R16	slow	rod	convex	mucous	smooth	-	White	Motile
R17	fast	spherical	convex	mucous	smooth	-	White	Motile
R18	slow	rod	convex	mucous	smooth	-	White	Motile
R19	slow	rod	convex	mucous	smooth	-	White	Motile
R20	slow	rod	convex	mucous	smooth	-	White	Not motile
R21	slow	rod	convex	mucous	smooth	-	White	Motile
R22	slow	rod	convex	mucous	smooth	-	White	Motile
R23	fast	rod	convex	mucous	smooth	-	White	Motile

2-Biochemical tests

Table 8 shows some biochemical tests for bacteria isolated from the root nodules of Faba bean plants, It was found that most of the isolates gave colors from pink, light pink and white when grown on the medium of the Congo red stain with a concentration of 0.025% according to its ability to absorb the stain, as it gave 5 isolates the pink color, while the rest of the isolates gave light pink and white color, and the reason for this is due to the low ability of the root nodule bacteria to absorb the stain which indicates the purity of the studied isolates, while other bacteria are colored in red, the same table shows the results of the 0.5% bromothymol blue stain test, which indicates the bacteria that are able to change the color of the medium from green to yellow, belonging to the fast-growing species such as *Rhizobium*, and the bacteria that change the color of the medium to blue belong to the slow-growing species such as the genus *Bradyrhizobium*., most of the isolates of the study gave yellow color for their production of compounds that increase the acidity of the medium after growing on this medium, while the isolates (R7, R16, R18, R19, R20, R21, R22) gave the blue color, which indicates their production of compounds that increase the basicity of the medium, and converge with the results Yesmin et al. (2021) in their study on *Rhizobium* bacteria isolated from the root nodules of Faba bean plants, and indicated that the bacterial isolates have the ability to produce compounds that increase the acidity of the medium and belonging to the fast-growing species after growing on this medium, The same table shows the results of the Hofer alkaline test, which shows that all bacterial isolates gave a negative result of the test, and this indicates their inability to grow at high levels of pH, which distinguishes them from other bacterial genera such as *Agrobacterium*, which have the ability to grow at these levels, and from the keto–Lactose test, all isolates gave a positive result except for two isolates that gave a negative result for the test, and this converges with Al-Samarrai (2017) when studying the root nodule bacteria isolated from several leguminous plants.

Table 8 indicates that 20 isolates have the ability to hydrolysis hydrogen peroxide into oxygen and water, while 3 isolates gave a negative result for the test. The oxidase test, all isolates gave a positive test result, as this result agrees with the results of Hewedy et al. (2015) in their study on *Rhizobium* bacteria isolated from the root nodules of Faba bean plants from different regions of

Egypt, they noticed that the bacterial isolates have the ability to produce the oxidase enzyme., As for the citrate test, it was found that all isolates gave a positive result, except for isolates R14 and R21, which gave a negative result for the test, This is due to the isolates' ability to consume citrate as a source of carbon, which leads to a change in the color of the medium from green to blue as a result of an increase in the pH of the medium. ,and show results the ability of all isolates to secrete urease enzyme that leads to hydrolysis of the urea to ammonia so the pH of the medium , leading to the transformation of the color of the medium to pink., This result agrees with the results of Hewedy et al. (2015) in their study on *Rhizobium* bacteria isolated from the root nodules of the Faba bean plant. to the gelatin test, most of the bacterial isolates gave a negative result for the test except for 5 isolates that gave a positive result, and this is similar to the result of Al-Samarrai (2017) in his study on root nodule bacteria isolated from root nodules and the soil surrounding the roots of leguminous plants, including cowpea and soybean., As for the triple-sugar iron test, most of the isolates gave their ability to ferment one or more of the three sugars present in the components of the medium, and other isolates gave their ability to produce oxygen gas bubbles, while most of the isolates gave their ability to produce hydrogen sulfide which gives black color, and this result agrees with the results of Al-Samarrai (2017) in his study on the root nodule bacteria, which gave all isolates a positive test result.

Table (8): Some biochemical tests of Rhizobium bacteria isolated from the root nodules of the Faba bean plant

Isolates	C.R	BTB	Keto-Lactose	Hofer	Catalase	Oxidase	Citrate	Urea	Gelatin	TSI
R1	light pink	Yellow	+	-	+	+	+	+	-	+
R2	pink	Yellow	+	-	+	+	+	+	-	+
R3	White	Yellow	+	-	+	+	+	+	-	+
R4	light pink	Yellow	+	-	-	+	+	+	-	+
R5	White	Yellow	+	-	+	+	+	+	+	+
R6	White	Yellow	+	-	-	+	+	+	-	+
R7	light pink	Blue	-	-	+	+	+	+	+	++
R8	light pink	Yellow	+	-	+	+	+	+	-	+
R9	pink	Yellow	+	-	+	+	+	+	+	++
R10	White	Yellow	+	-	+	+	+	+	-	++
R11	light pink	Yellow	+	-	+	+	+	+	-	+
R12	pink	Yellow	+	-	+	+	+	+	+	++
R13	light pink	Yellow	+	-	+	+	+	+	-	+++
R14	pink	Yellow	+	-	+	+	-	+	-	++
R15	light pink	Yellow	+	-	+	+	+	+	+	+
R16	pink	Blue	+	-	+	+	+	+	-	+
R17	White	Yellow	+	-	+	+	+	+	-	+
R18	White	Blue	-	-	+	+	+	+	-	++
R19	light pink	Blue	+	-	+	+	+	+	-	+++
R20	White	Blue	+	-	+	+	+	+	-	++
R21	light pink	Blue	+	-	+	+	-	+	-	+
R22	light pink	Blue	+	-	+	+	+	+	-	+
R23	light pink	Yellow	+	-	-	+	+	+	-	+

3-Biological activity of bacterial isolates

1-**Efficiency of isolates in producing indole acetic acid:** Figure 1 shows the ability of bacterial isolates to produce indole acetic acid, which shows that isolate R15 gave the highest efficiency in production of indole at a rate of 28.4 µg ml^{-1}, followed by isolates R19, R13 and R17 which gave a rate of The production of indole was 26.1, 24.5 and 21.9 µg ml^{-1}, respectively, and the isolate R23 gave the lowest ability to produce indole, which was 13.0µg ml^{-1}, this result agrees with the results of Matse et al. (2020) when they studied on strains belonging to *Rhizobium* bacteria, The isolates CHB1120 and CHB1121 of *Rhizobium* gave the highest efficiency of 13.3 and 13.8 µg ml^{-1} respectively.

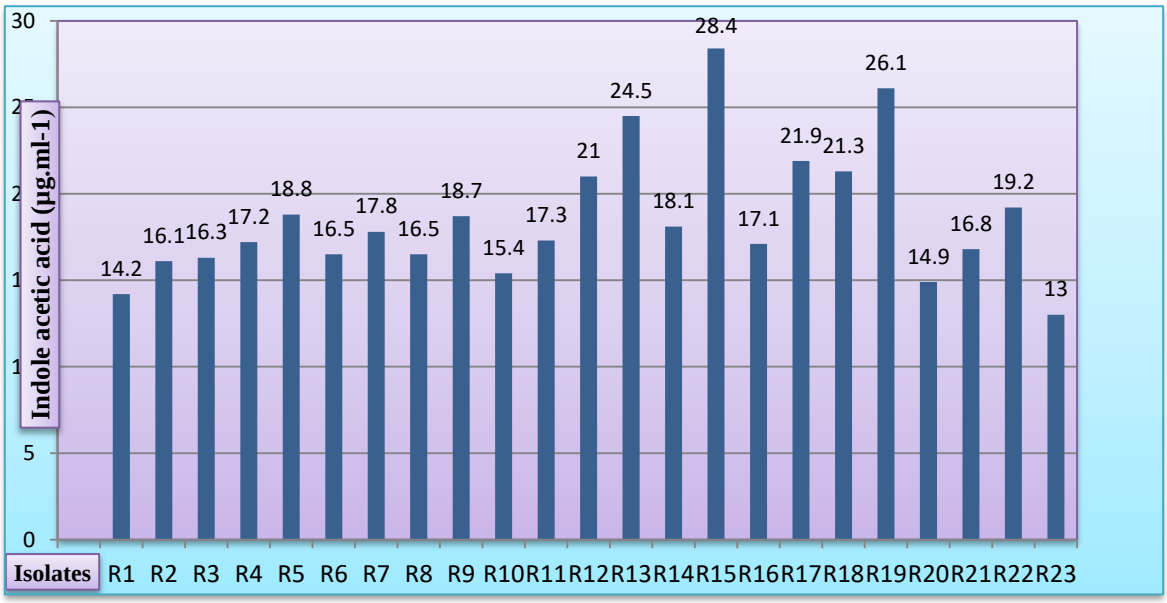

Figure (1): Efficiency of isolates of the family Rhizobiaceae to produce indole acetic acid

2- Efficiency of the isolates in producing chelating compounds: Table 9 shows the ability of isolates to produce iron chelating compounds, which shows that most bacterial isolates are able to grow in this medium,gave the isolates (R2, R9, R14, R17) a high chelation ability through their dense growth, while 13 isolates gave an average ability to chelate iron, while the two isolates R8 and R11 were unable to grow in this medium., the difference in isolates in their production of these compounds may be due to their difference in growth on this medium, bacteria that do not grow on this medium do not have the ability to produce chelating compounds and cannot withdraw iron from the medium, and the bacteria producing these compounds have the ability to withdraw this ion from compound 2.2dipyrdil, and binds to it and transports it into the bacterial cell to benefit from it in metabolic activities, and it agrees with Kumar et al. (2014) in their study on the Fenugreek plant .

Table (9): The ability of some isolates of Rhizobia bacteria to produce iron chelating compounds

Isolates	Iron chelating	Isolates	Iron chelating
R1	++	R13	++
R2	+++	R14	+++
R3	++	R15	++
R4	++	R16	++
R5	++	R17	+++
R6	+	R18	++
R7	++	R19	++
R8	-	R20	++
R9	+++	R21	+
R10	++	R22	+
R11	-	R23	+
R12	++		

4- Molecular identification results: The isolate RI-1was identificated molecularly under the world number KX397275.1 and the bacterial isolate name *Rhizobium leguminosarum bv. viciae strain SMV12a* , and the figure A2 shows the template of the DNA segment used in the amplification process, and Figure B2 shows the result of the PCR process after amplifying the DNA segment, and figure C2 shows the product of gel electrophoresis for extracting DNA from the isolate, as the isolate was identificated molecularly using a specialized primer (1250pb) according to (Miller et al

,2013), which targets ribosomal region 1087 in 16SrRNA which showed ribosomal region 61 to 1138 similarity of 99.69% with the bacterial species *Rhizobium leguminosarum bv. Viciae.*

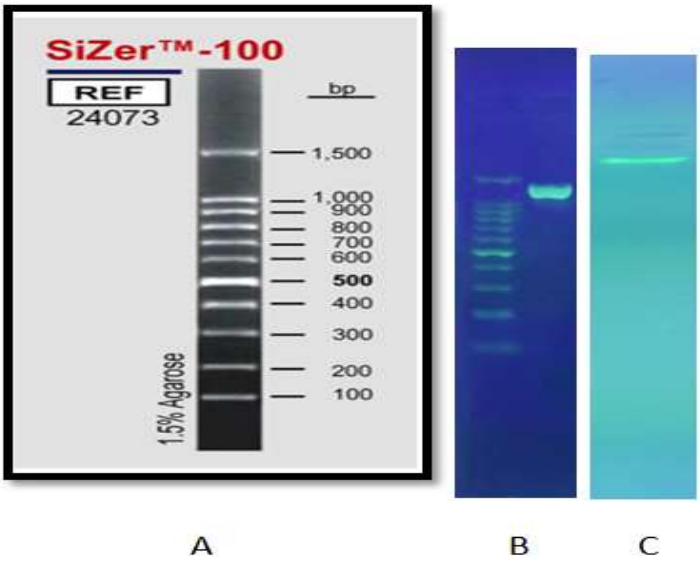

Figure (2): A - Template of the DNA fragment used in the amplification process
B - The result of the PCR process after the DNA fragment was amplified.
C – Gel electrophoresis product for DNA extraction from the isolation.

Table (10): Molecular characterization of bacterial isolates based on the percentage of matches of 16Sr RNA gene sequences with bacterial strains in the World Genebank

Code for isolate of the bacteria to be identificated	type of the bacteria the corresponds best	world number	Country	Similarity %	World number of recorded bacterial isolate
RI-1	*Rhizobium leguminosarum bv. viciae strain SMV12a*	KX397275.1	Germany	99.69	not recorded

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عزل وتشخيص بكتيريا الرايزوبيوم من جذور الباقلاء النامية في تربة جبسية واختبار كفاءتها في إنتاج منظم النمو (IAA) والمركبات المخلبية

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الخلاصة

خلال هذه الدراسة جُمعت 30 عينة من العقد الجذرية لنباتات الباقلاء (Vicia faba L.) النامية في تربة جبسية في كلية الزراعة / جامعة تكريت / محافظة صلاح الدين بتاريخ 5/12/2020 , وعزلت على الوسط الإختياري (مستخلص الخميرة وسكر المانيتول الصلب) وشُخصت العزلات النقية بالإعتماد على الصفات المزرعية والمجهرية والكيموحيوية ,وأظهرت نتائج التشخيص المظهري بان 23 عزلة أعطت صفات بكتيريا Rhizobium sp. ,إذ كان لون المستعمرات ما بين الأبيض والكريمي والأصفر ولماعة ومحدبة وملساء ,وسالبة لصبغة كرام وأغلبها متحركة وذات ألوان تتراوح ما بين الوردي الفاتح والأبيض على وسط صبغة الكونغو الحمراء بتركيز (0.025%) وكانت أغلبها سريعة النمو بإعطائها اللون الأصفر على وسط البروموثايمول الزرقاء وغير قادرة على النمو على وسط هوفر القلوي ,وأُختيرت العزلة الكفوءة في إنتاج منظم النمو والمركبات الخالبة للحديد وشُخصت جزيئياً بالإعتماد على تقنية PCR ,إذ تم تضخيم الجين SrRNA16 ثم تم تحليل تتابع القواعد النتروجينية وبينت النتائج بأن هناك تشابه 99.69% مع السلالة Rhizobium leguminosarum bv. Viciae strain SMV12a وعليه تعد العزلة قريبة وراثياً مع بكتريا Rhizobium .

الكلمات المفتاحية: بكتيريا الريزوبيوم ، التربة الجبسية ، التشخيص الجزيئي ، النباتات البقولية