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Comprehensive Morphological, Biochemical, and Molecular Characterization of *Azotobacter vinelandii* Isolated from Tomato (*Solanum lycopersicum*) Soils in Iraq and Assessment of Its Nitrogen-Fixation Efficiency

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Abstract:

This study involved the isolation and identification of eight *Azotobacter vinelandii* isolates from tomato plant soils, followed by morphological, biochemical, and molecular characterization using polymerase chain reaction (PCR). The morphological and biochemical properties of the A. Vinelandii isolates were examined using conventional methods, and these characteristics were consistent with those of Azotobacter spp. Molecular analysis confirmed the identification of the isolates obtained by traditional methods. The bacterial cells were pleomorphic, Gram-negative, motile, and positive for oxidase, catalase, gelatinase, Simon's citrate, Voges-Proskauer, methyl red, and indole tests. The isolates were capable of nitrate reduction and nitrogen fixation in nitrogen-free liquid medium (Nfb). All eight bacterial isolates exhibited varying nitrogen-fixation capacities, with isolate AZ1 demonstrating the highest efficiency among them. PCR analysis showed a 1250-bp band confirming the presence of A. Vinelandii. Sequencing results revealed 99% similarity with A. Vinelandii sequences in the genbank (NCBI) database. Phylogenetic analysis indicated that the Iraqi isolates are closely related evolutionarily to A. Vinelandii isolates from other countries, showing 99.76% similarity.

Keywords: *Azotobacter vinelandii*, biochemical, molecular, nitrogen fixation.

Introduction

Azotobacter spp, Can non-symbiotic, heterogeneous, free-living microorganisms fix nitrogen in soil at an average of 20 kg N per annum at the atmospheric level? They further increase nutrient absorption and release bioactive compounds of plants including amino acids and proteins serving as plant growth-promoting rhizobacteria (PGPR) by releasing growth-promoting chemicals that can promote plant growth and protect against phytopathogens by the production of inhibitory compounds.(1)

The application of molecular techniques in this study was based on the significance of these bacterial isolates and the fact that the technique is more accurate in the identification of bacterial strains that can be utilized to soil and plants as bioinoculants.(2)

Azotobacteraceae family is broken down into two major genera namely Azotobacter and Azomonas according to various attributes. Azotobacter genus comprises six common species of the genus: A. Beijerinckii, A. Chroococcum, A. Nigricans, A. Vinelandii, A. Armeniacus and A. Paspali. The most common of these are A. Vinelandii and A. Chroococcum that are common in the Iraqi soils.(4 ,3)

Morphologically, in case of Azotobacter spp. Grow on nitrogen-free agricultural media which include the use of sucrose or alcohol as a source of carbon, their colonies are generally large, spreading, smooth, opaque, homogeneous and shiny though the colonial characteristics of different colonies differ depending on the media and carbon sources.(6 ,5)

A. Vinelandii has heteromorphic morphological characteristics; it can be inhabited by rod- or oval-shaped cells, with a mature cell measuring 23 mm in size. They are flagellar, obligately aerobic, fix nitrogen and release plant hormones including IAA and vitamins. They are also known to generate biopolymers like alginates and antioxidant compounds so that they can withstand stress and therefore can be used as biofertilizers to improve plant growth. The bacterial isolates are capable of using mannitol, sucrose, and starch as carbon and energy sources, grow in 1 percent sodium benzoate medium, and can resist thermal up to 37 o C.(7)

The morphological features of Azotobacter that grew on nitrogen-free Mannitol agar in a laboratory study were dark brown to green, convex, viscous, and uneven margins. The microscopic appearance of the cells was flattened to rounded, Gram-negative, and motile.(8)

Molecular research on Azotobacter revealed about 50 strains of soil and recognized three species namely A. Chroococcum, A. Vinelandii and A. Beijerinckii. The primers used to amplify the 16S rrna gene were 27F and 1495R after which they were digested with Hpall, Rsal and Hhal restriction enzymes. Restriction analysis demonstrated that there was polymorphism within the species with A. Vinelandii being more heterogenous than the other two species.(9)

The conventional identification of Azotobacter using the morphological features is tedious and time consuming. Thus, different molecular techniques are currently being used to identify these bacteria within a short time with high precision.(4 ,10)

The present research will isolate Azotobacter vinelandii found in soil and describe it morphologically, biochemically, and molecularly.

Materials and Methods

Isolation *of Azotobacter vinelandii*: *Azotobacter vinelandii* were isolated using eight soil samples of the rhizosphere of the plants in the various regions of the fields in Babil Governorate as indicated in Table 1. The isolation and identification of A. Vinelandii were also done on a representative sample of each field. The samples labeled were put in sterile plastic bags and refrigerated until usage.

Isolation of bacteria was through the serial dilution procedure. The soil suspension was prepared by putting ten grams of soil in 250 ml conical flask with sterile distilled water (10 grams). A thorough mix of the suspension was done, and serial decimal dilutions ranging between 10 -1 and 10 -6 were made by transferring 1 ml of the soil suspension to test tubes holding 9 ml of sterile Nfb medium. The samples of soils were made in three copies. Incubation of the tubes was done at 30 o C in 72 hours. The presence of Azotobacter was indicated by formation of a ring or a pellicle on the surface.Some of the growth was then subculture to specialized solid media to purify the colonies and examine their cultural properties in 3-5 days. A further purification of each colony was done by streaking on the same medium to ascertain purity. The isolates were designated codes and numbers by the place where they were isolated and kept until subsequent diagnostic procedures were conducted.

Table 1. Number and code of isolates, source, and collection location

No.	Isolate Code	Source of Isolation	Location
1	AZ1	Tomato field	Babil – Alkifl
2	AZ2	Eggplant field	Babil – Al-Musayyib Project
3	AZ3	Pepper field	Babil – Nahiyat Al-Nil
4	AZ4	Potato field	Babil – Nahiyat Al-Nil
5	AZ5	Eggplant field	Babil – Abu Ghraq
6	AZ6	Tomato field	Babil – Abu Ghraq
7	AZ7	Pepper field	Babil – Al-Musayyib Project
8	AZ8	Eggplant field	Babil – Alkifl

Identification of A. Vinelandii Isolates by Conventional Methods

Cultural Properties: The cultural properties of the generated colonies in Azotobacter vinelandii medium that were specific to the cultures of the colonies were documented as per the procedure outlined in (11). Colony shape, texture, transparency, growth density, colony size, margin shape, surface appearance, optical characteristics, colony elevation and color were among the observations.

Microscopic Properties: Gram staining was done followed by microscopic observation of the cell morphology and staining reaction as per the method described by.(11)

Biochemical Tests: A number of biochemical tests were performed to determine the viability and metabolic properties of A. Vinelandii, such as, growth on 0.1% phenol and 1% sodium chloride (12), catalase test (13), oxidase test (14), urease test, Vibrio motility test (15), indole test (16), as well as Gelatin hydrolysis test.(17)

Molecular Identification Polymerase Chain Reaction (PCR), sequencing and Phylogenetic Analysis.

PCR Analysis: The primers were amplified by PCR with the primers described in Table **2.**

Table 2. Sequences of primers used in this study

Primer	Sequence	Primer sequence 5'- - 3'	Tm (°C)	GC(%)	Size of Product (bp)
16s RNA	**F**	**AGAGTTTGATCCTGGCTCAG**	**54.3**	**50.0**	**1250**
	R	**GGTTACCTTGTTACGACTT**	**49.4**	**42.1**	

DNA Extraction: DNA genomic DNA of pure cultures was extracted using the favorprep Total DNA Mini Kit following the protocol of their manufacturer (FAVORGEN, Korea) as seen in Table 3.

Table 3. Components of the DNA extraction kit used in this study

Cat. No:	FABGK 100 (100 preps)		
RBC Lysis Buffer	135 ml		
FATG Buffer	30 ml		
FABG Buffer	40 ml		
W 1 Buffer	45 ml		
Wash Buffer * (concentrate)	25 ml		
Elution Buffer	30 ml		
FABG Mini Column	100 pcs		
Collection Tube	200 pcs		
User Manual	1		
Preparation of Wash Buffer by adding ethanol (96 ~ 100%)			
* Ethanol volume for Wash Buffer	4 ml	100 ml	200 ml

- PCR amplification was performed using a PCR system following the steps outlined in Table 3, under the specified thermal cycling conditions.

Table 3. Steps of the PCR reaction

No.	Phase	Tm (°C)	Time	No. Of cycle
1-	Initial Denaturation	95°C	3 min	1 cycle
2-	Denaturation -2	92°C	45 Sec	30 cycles
3-	Annealing	66°C	45 Sec	
4-	Extension-1	72°C	45 Sec	
5-	Extension -2	72°C	7 min.	1 cycle

- A portion of the bacterial samples was finally stored at 4°C or 20°C until further use.

Electrophoresis of PCR Products:

- The product of the PCR was electrophoresed using 1 X running buffer and 0.5X loading dye.
- Agarose solution Agarose solution was made by dissolving 1 g of agarose powder in 100 ml of 1X TAE buffer in a 100 ml flask. This solution was heated on a hot plate till it became clear.
- The agarose solution was allowed to cool to a temperature of about 50-55 C with some light swirling of the solution to create even cooling.
- Agarose solution was cooled and then 3 µl of red stain was poured on it.
- The PCR product was combined with 3 µl of DNA loading buffer on a clean glass plate (2 ml).
- The mixture was applied to the agarose gel wells with a micropipette that was precise.
- Electrophoresis of the samples was performed at 80 V and 65 ma (1 hour).
- DNA bands were observed under UV light using UV transillluminator.

Sequence Analysis: PCR products of two bacterial isolates were forwarded in Korea to purify and sequence. The Sanger sequencing was done with bacterial-specific primers.

Phylogenetic Analysis: Phylogenetic trees of the two bacterial isolates were drawn using a comparison of the sequences with the nearest sequence that had been found within the genbank database. It was done using the Maximum Composite Likelihood method, which was adopted in MEGA6 software.

Nitrogen-Fixation Ability of Bacterial Isolates: The fixation of atmospheric nitrogen ability of A. Vinelandii isolates was conducted as per the procedure outlined by (18). The bacteria were cultured in a bacterial culture in nitrogen free liquid media (Nfb) with a carbon source of sucrose and a mineral salt nitrogen free source. The medium (50 ml) was put in 250 ml flasks, 1% Mannitol solution was added to it, and 2 ml of bacteria culture in each isolate was added. The incubation period of the flasks was seven days in a shaking incubator at 30 37C. The quantity of ammonia synthesized in the medium was calculated by adding 10 ml of the culture into the Kjeldahl method**.**

Results and discussion

Cultural and Microscopic Characteristics of Azotobacter vinelandii: The findings of Table 4 represent some cultural and microscopic features of Azotobacter isolates collected on the rhizosphere soils of several vegetable crops (Table 1). Under microscopic observation, the bacterial cells were observed to be pleomorphic and they were either rods or spherical in shape, Gram-negative, and motile.

All the isolates were capable of fixing atmospheric nitrogen in nitrogen-free liquid medium (Nfb), in the form of a thin brown pellicle on the surface of the medium. The colonies which grew in the solid media were mostly viscous, convex, semi-opaque, and shiny. The surfaces of the colonies were mostly smooth and the margins were either complete or wavy. Colony sizes were medium to large, the density of growth of bacteria was medium to abundant, the colony shape was circular, and the color was brown to yellow.

These findings are in agreement with the report made by (19). The characteristics of these isolates, including these cultural and microscopic features, are consistent with the common features of Azotobacter species since it is based on the previous investigations and the observed nature of these isolates.

Table 4. Cultural and microscopic characteristics of *Azotobacter vinelandii* isolates

Characteristic	AZ1	AZ2	AZ3	AZ4	AZ5	AZ6	AZ7	AZ8
Colony shape	Circular	Circular	Circular	Irregular	Circular	Irregular	Circular	Circular
Colony margin	Entire	Entire	Entire	Entire–Wavy	Entire	Entire	Wavy	Entire
Colony surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Colony color	Brown	Brown	Brown	Yellow/Red	Brown	Brown	Brown	Yellow/Red
Colony elevation	High–Convex	High–Convex	Convex	Convex	Highly Convex	Convex	High–Convex	Highly Convex
Colony size	Large	Large	Large	Large–Medium	Large–Medium	Large	Large–Medium	Large
Optical characteristics	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny	Shiny
Transparency	Semi-opaque	Semi-opaque	Semi-opaque	Semi-opaque	Semi-opaque	Semi-opaque	Semi-opaque	Semi-opaque
Colony consistency	Very viscous	Very viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous
Bacterial growth density	High	High	High	Medium	High	High	Medium	High
Cell shape	Rod	Rod	Rod	Rod–Coccus	Rod	Rod	Rod	Rod–Coccus
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Cell motility	Motile	Motile	Motile	Motile	Motile	Motile	Motile	Motile

Biochemical Tests: Table 5 presents the results of some of the diagnostic biochemical tests that are conducted to determine the viability and metabolic properties of the bacterial isolates. All the isolates were capable of growing on NA medium and were motile with positive oxidase, catalase and gelatinase test. They also were positive in the use of citrate, Voges- Proskauer (VP) and methyl red tests, and were able to reduce nitrate to nitrite.The isolates could grow in 0.1% phenol and nacl 1%. They were able to grow at 4o C and not at 42 o C. All the isolates were positive in indole production but failed to use starch as a source of carbon and negative in urease activity.

According to the earlier researches on Azotobacter spp., the biochemical and cultural properties are in line with the characteristic features of the Azotobacter species. Based on the cultural, microscopic and biochemical test findings as presented in Table 5, the bacterial isolates all eight had the typical characteristics of A. Vinelandii. This observation is in line with the findings that (20) observed, which all the isolates were A. Vinelandii as it is indicated in Table 5.

Table 5. Biochemical characteristics of *Azotobacter vinelandii* isolates

Test	AZ1	AZ2	AZ3	AZ4	AZ5	AZ6	AZ7	AZ8
Gram stain	–	–	–	–	–	–	–	–
Motility test	+	+	+	+	+	+	+	+
Voges-Proskauer (VP) test	+	+	+	+	+	+	+	+
Starch hydrolysis	–	–	–	–	–	–	–	–
Nitrate reduction (NO_3^-)	+	+	+	+	+	+	+	+
Indole test	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+
Urease test	–	–	–	–	–	–	–	–
Methyl Red test	+	+	+	+	+	+	+	+
Gelatin hydrolysis test	+	+	+	+	+	+	+	+
Citrate utilization test	+	+	+	+	+	+	+	+
Growth at 4°C	+	+	+	+	+	+	+	+
Growth at 42°C	–	–	–	–	–	–	–	–
Nacl 1%	+	+	+	+	+	+	+	+
Phenol 0.1%	+	+	+	+	+	+	+	+

Molecular Identification of A. Vinelandii Isolates

PCR Results: Following the selection of eight A. Vinelandii isolates whose identification was made based on cultural, microscopic and biochemical appearances, the isolates were given numbers AZ1, AZ2, AZ3, AZ4, AZ5, AZ6, AZ7, and AZ8. Genetic identification of these isolates was done in part by the pair of A. Vinelandii-specific 16S rrna gene primers (16S rrna Forward and 16S rrna Reverse) using PCR.

Initial PCR findings revealed that all isolates had clear DNA bands (Figure 1), which was a measure of the validity of the DNA extraction technique. A selection of distinct bands was formed by the bacterial strains of about 1250 base pairs of the 16S rrna gene, which is the anticipated size that would be amplified by this primer pair in A. Vinelandii DNA. The primers were able to anneal to the complementary sequences of the bacterial DNA template and not anneal to the non-target sequences, as established through the agarose gel electrophoresis. These findings affirmed that all the eight isolates are of the species A. Vinelandii.

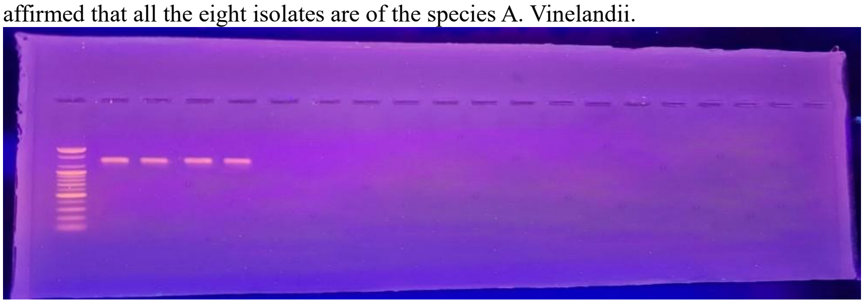


Photo 1. DNA products extracted from A. Vinelandii isolates and separated on 1.5% agarose gel.

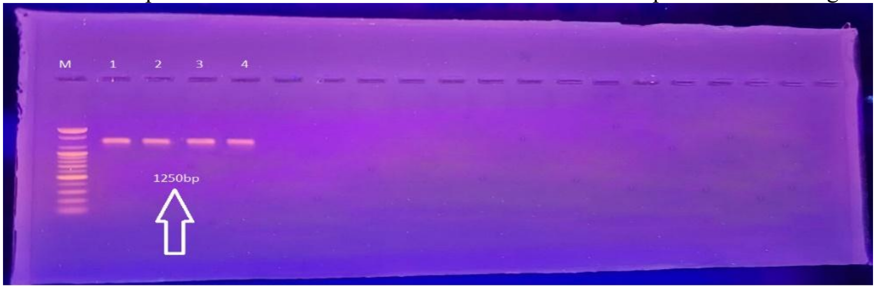


Photo2. PCR amplification products of a 100 bp DNA fragment of *Azotobacter vinelandii*, separated on 1.5% agarose gel. Lane M represents the DNA ladder, lanes 1–4 indicate isolates positive for the target gene, whereas lanes 5–8 represent isolates negative for the gene.

Sequence Analysis of Amplified PCR Products: Once A. Vinelandii was identified by means of PCR with specially designed primer specific to this bacterial species and the appearance of clear amplification bands, the two isolates were forwarded to Microgen Company (South Korea) to determine the sequence (Table 6).The analysis of sequences also indicated that the first isolate had a variation of 1 percentage at transversion sites (393, 677, and 885) with a C/A, C/G, and T/G nucleotide substitutions respectively leading to a similarity of 99. The second isolate had a 1 percent difference at the transversion sites (363 and 730), with the nucleotide changes of C/A and T/A, respectively and resulted in a 99 percent parallels with the sequences of A. Vinelandii in the genbank (NCBI) database as in Table 7.

Isolates were also deposited in the genbank database, as A. Vinelandii Am. Ja.3, as indicated by NCBI records (Appendix 8) and were given accession number PV786135.

Table 6. Sequence analysis results of two A. Vinelandii isolates

16S ribosomal RNA gene

No. Of sample	**Type of substitution**	**Location**	**Nucleotide**	**Source**	**Sequence ID with compare**	**Sequence ID with submission**	**Identities**
1	**Transvertion**	363	C\A	***Azotobacter vinelandii***	**ID: MN340239.1**		**99%**
	Transvertion	677	C\G				
	Transvertion	885	T\G				
2	**Transvertion**	363	C\A	***Azotobacter vinelandii***	**ID: MN340239.1**		**99%**
	Transvertion	730	T\A				

Azotobacter vinelandii strain SKUDBTABF101 16S ribosomal RNA gene, partial sequence
Sequence ID: MN340239.1Length: 1370Number of Matches: 1
Range 1: 84 to 923genbankgraphicsnext matchprevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1502 bits(1665)	0.0	837/840(99%)	0/840(0%)	Plus/Plus

Azotobacter vinelandii strain SKUDBTABF101 16S ribosomal RNA gene, partial sequence
Sequence ID: MN340239.1Length: 1370Number of Matches: 1
Range 1: 84 to 923genbankgraphicsnext matchprevious Match

Alignment statistics for match #1				
Score	Expect	Identities	Gaps	Strand
1507 bits(1670)	0.0	838/840(99%)	0/840(0%)	Plus/Plus

Phylogenetic Analysis of A. Vinelandii: MEGA6 software identified the evolutionary analysis of the final dataset based on the evolutionary analysis of the dataset as outlined in (21) to calculate the genetic associatedness and divergence to the global isolates and Iraqi isolates. The findings indicated that genetic similarities between the Iraqi isolates (1 and 2) having accession numbers of PV786135 and PV786136 were 99.76 and 99.64, respectively.

The UPGMA method was used to infer the evolutionary history of the isolates of the bacteria (22). The best tree derived was that with a total length of the branches of 0.31202221 and was disengaged, with the length of the branches expressed in the same units of evolution distance used in the development of the phylogenetic tree.

It was done using the Maximum Composite Likelihood approach outlined by (23) whereby the units are the number of base substitutions per site. This was in the form of an analysis of 15 nucleotide sequences and codon positions factor in as 1 st + 2 nd + 3 rd + noncoding regions. All positions containing gaps and missing data were eliminated, resulting in a final dataset comprising 839 positions.

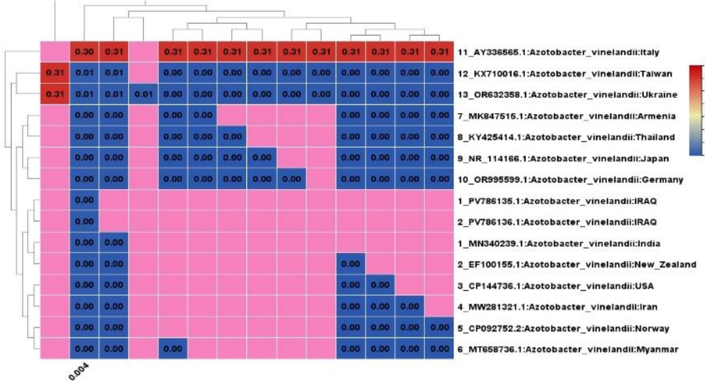


Figure 1. Phylogenetic tree analysis of *Azotobacter vinelandii* isolates.

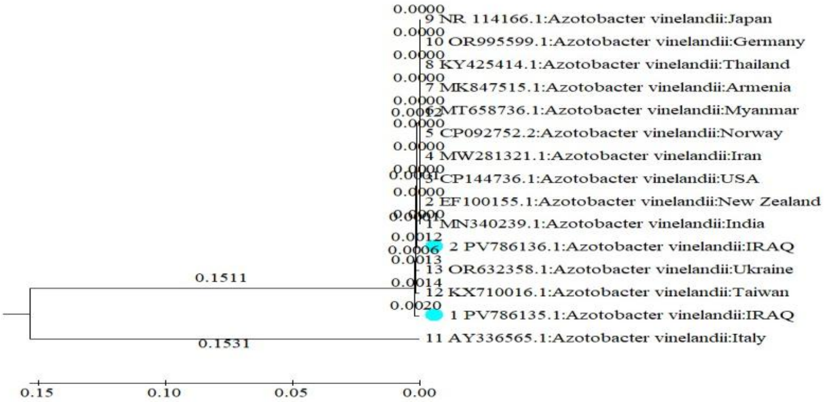


Figure 2. Phylogenetic tree results of *Azotobacter vinelandii* isolates.

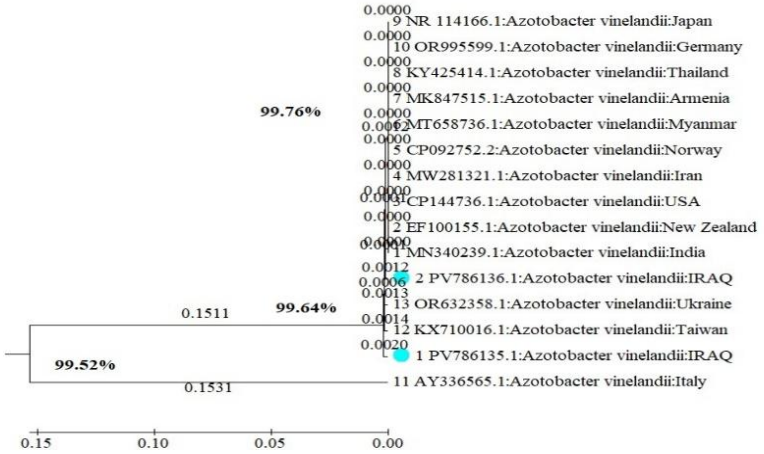


Figure 3. Phylogenetic tree results of *Azotobacter vinelandii* isolates.

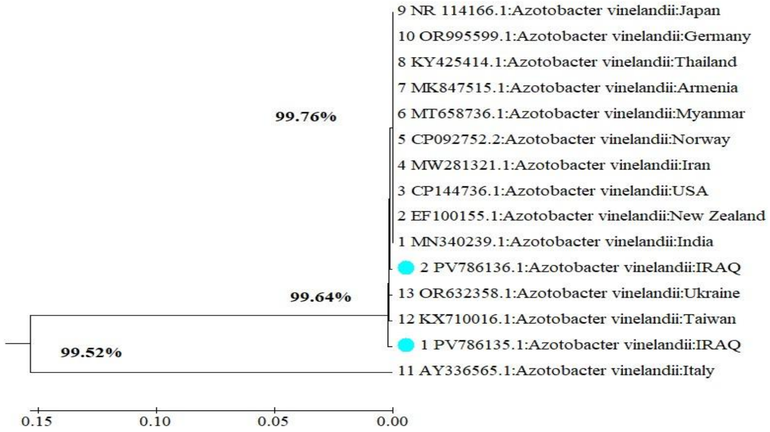


Figure 4. Phylogenetic tree analysis of *Azotobacter vinelandii* isolates.

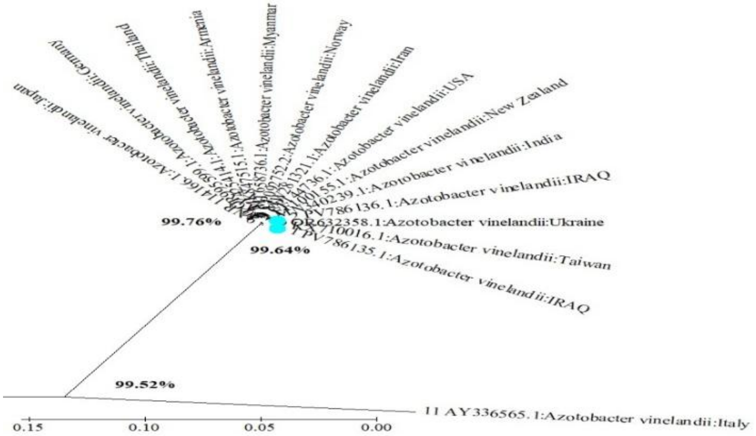
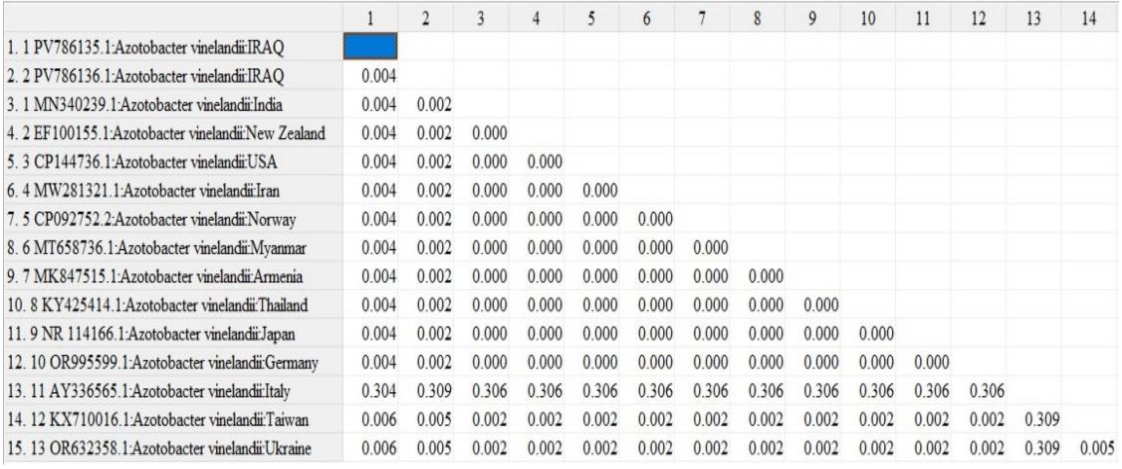


Figure 5. Final phylogenetic tree of *Azotobacter vinelandii* isolates.

Table 7. *Azotobacter vinelandii* isolates from different countries listed in the genbank database.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. 1 PV786135.1:Azotobacter vinelandii:IRAQ	🟦													
2. 2 PV786136.1:Azotobacter vinelandii:IRAQ	0.004													
3. 1 MN340239.1:Azotobacter vinelandii:India	0.004	0.002												
4. 2 EF100155.1:Azotobacter vinelandii:New Zealand	0.004	0.002	0.000											
5. 3 CP144736.1:Azotobacter vinelandii:USA	0.004	0.002	0.000	0.000										
6. 4 MW281321.1:Azotobacter vinelandii:Iran	0.004	0.002	0.000	0.000	0.000									
7. 5 CP092752.2:Azotobacter vinelandii:Norway	0.004	0.002	0.000	0.000	0.000	0.000								
8. 6 MT658736.1:Azotobacter vinelandii:Myanmar	0.004	0.002	0.000	0.000	0.000	0.000	0.000							
9. 7 MK847515.1:Azotobacter vinelandii:Armenia	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000						
10. 8 KY425414.1:Azotobacter vinelandii:Thailand	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000					
11. 9 NR 114166.1:Azotobacter vinelandii:Japan	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
12. 10 OR995599.1:Azotobacter vinelandii:Germany	0.004	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000			
13. 11 AY336565.1:Azotobacter vinelandii:Italy	0.304	0.309	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306	0.306		
14. 12 KX710016.1:Azotobacter vinelandii:Taiwan	0.006	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.309	
15. 13 OR632358.1:Azotobacter vinelandii:Ukraine	0.006	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.309	0.005

Table 8. Matching results of isolates using the Maximum Likelihood method.

	Azotobacter vinelandii ; 16S ribosomal RNA gene				
	Accession	Country	Isolation Source	Date of registration	Compatibility
1	ID: MN340239.1	India	Soil	2019	99%
2	ID: EF100155.1	New Zealand	--------------	2006	99%
3	ID: CP144736.1	USA	--------------	2023	99%
4	ID: MW281321.1	Iran	Saline lake	2020	99%
5	ID: CP092752.2	Norway	Soil	2024	99%
6	ID: MT658736.1	Myanmar	--------------	2020	99%
7	ID: MK847515.1	Armenia	--------------	2019	99%
8	ID: KY425414.1	Thailand	Field	2017	99%
9	ID: NR_114166.1	Japan	--------------	2011	99%
10	ID: OR995599.1	Germany	--------------	2023	99%
11	ID: AY336565.1	Italy	--------------	2003	99%
12	ID: KX710016.1	Taiwan	--------------	2016	99%
13	ID: OR632358.1	Ukraine	Soil	2023	99%

Assessment of Nitrogen-Fixation Efficiency of Bacterial Isolates: The findings in Table 9 demonstrate that all the eight identified bacterial isolates were able to fix atmospheric nitrogen though at different intensities. Isolate AZ1, which was an isolate of A. Vinelandii, had the highest efficiency in regard to nitrogen-fixation when nitrogen-free Nfb was used and the fixation was 16.0 mg N when compared with other isolates of the same species.

It can be possible that the high nitrogen-fixation capability of the isolate can be explained by the high activity in Iraqi soils, the location of isolation, the environmental conditions, and the crop cultivated. These results are consistent with the past reports.(25 ,24)

Nitrogen fixation ability of A. Vinelandii is associated with its synthesis of bioactive compounds, such as enzymes, organic and inorganic acids and plant hormones, which increase the supply of nitrogen in the atmosphere. According to these findings, in this study the preparation of a biofertilizer was done using AZ1 as evidenced by Table 9.

Table 9. Amount of nitrogen fixed by A. Vinelandii isolates

Isolate Code	Fixed Nitrogen (mg L^{-1})
AZ1	16.0
AZ2	15.3
AZ3	14.5
AZ4	10.5
AZ5	12.5
AZ6	9.9
AZ7	13.0
AZ8	11.4

Conclusions

According to the findings of the current research, eight Azotobacter vinelandii isolates were successfully isolated in soils of various fields of various vegetable gardens at Babil Governorate in Iraq. All isolates showed the common morphological and microscopic features of the genus Azotobacter, they are Gram-negative, motile, and fix nitrogen. Biochemical laboratory tests revealed positive catalase, oxidase, gelatinase, Vibrio methylred, Vibrio indole, and Vibrio utilization of nitrate reduction and growth at 0.1 percent phenol, 1 percent nacl, and 4 o C, but not at 42 o C. PCR was used to identify the isolates through 16S rrna gene and this showed the presence of a 1250-bp amplicon, identifying all the isolates as A. Vinelandii. The analysis of sequences showed that it was 99 percent similar to reference sequences in the NCBI database with slight mutations in nucleotides, and structural evolution showed high relatedness (99.64–99.76) between the Iraqi isolates and other global A. Vinelandii isolates. They all had the capacity to fix atmospheric nitrogen with isolate AZ1 showing the highest efficiency (16 mg L^{-1}) and was thus a good candidate to develop biofertilizer. The nitrogen-fixation capacity of such isolates is linked to the synthesis of bioactive substances, which are enzymes, organic and inorganic acids, and plant hormones which increase nitrogen intake in plants. These results affirm that the Iraqi isolates of A. Vinelandii have morphological, biochemical and molecular characteristics that can be used to enhance the soil fertility and promote crop growth.

References

Abd Ali, H.A.A.A., 2025. Molecular study of Azotobacter bacteria and organic residues effects on some soil properties and maize growth. Ph.D. Thesis, College of Agriculture, University of Al-Qadisiyah, Iraq.

Ali, H.A.A. and J.A.K. Kamal, 2025. The effect of organic manure type and molecularly identified Azotobacter bacteria on some soil properties. *IOP Conf. Ser.: Earth Environ. Sci.,* 1487(1): 012207. https://iopscience.iop.org/article/10.1088/1755-1315/1487/1/012207

Al-Mousawi, M.A.A.M. and K.S. Jaber, 2014. Evaluation of Azotobacter vinelandii and some chemical compounds in controlling charcoal rot disease in cowpea caused by Macrophomina phaseolina under laboratory and field conditions. *Iraqi J. Agric. Sci.,* 19(5): 138-153. https://www.iasj.net/iasj/article/91586

Baron, E.J. and S.M. Finegold, 1990. Bailey and Scott's Diagnostic Microbiology. St. Louis: C.V. Mosby Company.

Black, C.A., 1965. Methods of soil analysis part 2: Chemical and microbiological properties. Madison, WI: Am. Soc. Agron.

Bremner, J.M., 1965. Total nitrogen. In: Methods of soil analysis: Part 2 chemical and microbiological properties. Madison, WI: Am. Soc. Agron., pp: 1149-1178.

Cappuccino, J.G. and C.T. Welsh, 2020. Microbiology: A Laboratory Manual. 12th Edn., Pearson.

Cappuccino, J.G. and N. Sherman, 2013. Microbiology: A Laboratory Manual. 10th Edn., Pearson Education.

Collee, J.G., R.S. Miles and B. Watt, 1996. Tests for the identification of bacteria. In: Collee, J.G., B.P. Marmion, A.G. Fraser and A. Simmons (Eds.), Practical Medical Microbiology. 14th Edn., New York: Churchill Livingstone, pp: 132-149.

Harrigan, W.F. and M.E. McCance, 1976. Laboratory Methods in Food and Dairy Microbiology. London: Academic Press.

Jain, D., J. Sharma, G. Kaur, A.A. Bhojiya, S. Chauhan, V. Sharma and E. Maharjan, 2021. Phenetic and molecular diversity of nitrogen-fixating plant growth promoting Azotobacter isolated from semiarid regions of India. *Biomed. Res. Int.,* 2021: 618-628. https://doi.org/10.1155/2021/6635752

Jnawali, A.D., R.B. Ojha and S. Marahatta, 2015. Role of Azotobacter in soil fertility and sustainability–a review. *Adv. Plants Agric. Res.,* 2(6): 1-5. https://medcraveonline.com/APAR/APAR-02-00069

Kozieł, M. and A. Gałązka, 2019. Characteristics of selected molecular methods used in identification and assessment of genetic diversity of bacteria belonging to the genus Azotobacter. *Pol. J. Agron.,* 38: 37-45. https://doi.org/10.26114/pja.iung.388.2019.38.04

Manik, M.B.S., 2023. Evaluation of MPKV's bacterial consortium on growth and yield of tomato crop. Doctoral Dissertation, Mahatma Phule Krishi Vidyapeeth.

Matloob, A.A.H., 2012. Identification of causes of bean root and stem rot and evaluation of some biological control agents for resistance. Ph.D. Thesis, University of Baghdad. https://iqdr.iq/search?query=Matloob+2012

Mazinani, Z. and A. Asgharzadeh, 2014. Genetic diversity of Azotobacter strains isolated from soils by amplified ribosomal DNA restriction analysis. *Cytol. Genet.,* 48: 293-301. https://doi.org/10.3103/S009545271405006X

Mukhtar, H., H. Bashir, A. Nawaz and I. Haq, 2018. Optimization of growth conditions for Azotobacter species and their use as biofertilizer. *J. Bacteriol. Mycol.,* 6: 274-278. https://medcraveonline.com/JBMOA/JBMOA-06-00214

Murumkar, D.R., S.G. Borkar and V.P. Chimote, 2012. Diversity of cell morphology, nitrogenase activity and DNA profile of Azotobacter isolates from soils of Maharashtra. *Bioinfolet,* 9(4b): 851-858.

Muthukumar, A., G.M. Sandhya and G. Dakshayini, 2021. Morphological and biochemical characterization–a comparative analysis of non-commercial and commercial plant growth promoting microorganisms. *Int. J. Curr. Microbiol. Appl. Sci.,* 10(2): 867-874. https://doi.org/10.20546/ijcmas.2021.1002.102

Patil, S.V., B.V. Mohite, C.D. Patil, S.H. Koli, H.P. Borase and V.S. Patil, 2020. Azotobacter. In: Beneficial Microbes in Agro-Ecology. Berlin: Springer, pp: 397-426. https://link.springer.com/chapter/10.1007/978-981-15-3208-5_15

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. San Francisco: Freeman.

Tamura, K., G. Stecher, D. Peterson, A. Filipski and S. Kumar, 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol. Biol. Evol.,* 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

Tamura, K., M. Nei and S. Kumar, 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA,* 101: 11030-11035. https://doi.org/10.1073/pnas.0404206101

Tchan, Y.T. and N.B. Peter, 1984. Genus Azotobacter. In: Sneath, P.H., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.), Bergey's Manual of Systematic Bacteriology. London: William and Wilkins, pp: 219-229.

Thompson, J.P. and V.B. Skerman, 1979. Azotobacteraceae. London: Academic Press.