Libyan Journal of Ecological & Environmental Sciences and Technology
(LJEEST)

LJEEST
ISSN: 2710-5237
www.srcest.org.ly/jou

DOI: https://doi.org/10.63359/0tsm3a41

Effect of Different Cultivation on Quantity and Activity of *Azotobacter Sp.* in Soil

Khunaw Abdullah Rahman

ARTICLE INFO

Vol. 7 No. 1 April, 2025

Pages (29 - 38)

Article history:

Revised form 1 December 2024
Accepted 13 January 2025

Authors affiliation
Salahaddin University-Erbil
College of Agriculture Engineering sciences.Soil & water department
Khunaw.rahman@su.edu.krd

Keywords:
Azotobacter spp., Abundance, Kurdistan Location, Molecular Analysis, Soil fertility

ABSTRACT

Farmers are facing challenges with cultivating different crops because of the continuous impact of calcareous soil on microbial activities dynamics. The key objective was to identify and quantify Azotobacter spp in cultivated and uncultivated soils across different locations and analyse their phylogenetic relationships based on partial 16S rRNA gene sequences. Soils were sampled from diverse crop locations and subjected to bacterial diversity analysis using the Maximum Likelihood method. The abundance and diversity of Azotobacter varied significantly between cultivated and uncultivated soils. The cultivated soils showed a higher prevalence of Azotobacter chroococcum in all locations, while uncultivated soils contained other species such as Azotobacter vinelandii and Azotobacter salinestris. The phylogenetic analysis confirmed the close genetic links among Azotobacter spp. as well as their unique clustering in relation to other genera such as Bacillus and Xanthobacter. The 16S rRNA sequencing phylogenetic tree showed that the identified Azotobacter species and other nitrogen-fixing taxa such as Xanthobacter, Pseudomonas, and Klebsiella, have close evolutionary links. The findings also showed that improved nitrogen fixation and cation exchange characteristics were indications of increased Azotobacter activity in the cultivated soils. This prevalence of Azotobacter in cultivated soils indicated their ability for adaptability in agricultural lands. The study concludes that cultivation practices significantly influence Azotobacter populations, favouring species with high nitrogen-fixing efficiency. Enhancing Azotobacter activity in soil can improve nitrogen availability and soil fertility. It is recommended to adopt crop rotation, organic amendments, and reduced chemical fertilizer use to sustain Azotobacter populations and maintain soil health. Hence, farmers and researchers in the Kurdistan area of Erbil are advised to use biofertilizers based on Azotobacter on calcareous soils to increase soil fertility and microbial activity.

تأثير أنماط الزراعة المختلفة على كمية ونشاط جنس Azotobacter في التربة

خوناو عبدالله رحمن

يواجه المزارعون تحديات في زراعة المحاصيل المختلفة بسبب التأثير المستمر للتربة الجيرية على ديناميكيات الأنشطة الميكروبية. الهدف الرئيسي هو تحديد وقياس Azotobacter spp في التربة المزروعة وغير المزروعة عبر مواقع مختلفة وتحليل علاقاتها التطورية بناءً على تسلسلات جينات 16 S rRNA الجزئية. تم أخذ عينات من التربة من مواقع محاصيل متنوعة وخضعت لتحليل التنوع البكتيري باستخدام طريقة أقصى احتمال. تباينت وفرة وتنوع Azotobacter بشكل كبير بين التربة المزروعة وغير المزروعة. أظهرت التربة المزروعة انتشارًا أعلى لـ Azotobacter chroococcum في جميع المواقع، بينما احتوت التربة غير المزروعة على أنواع أخرى مثل Azotobacter vinelandii و Azotobacter salinestris. أكد التحليل التطوري الروابط الوراثية الوثيقة بين Azotobacter spp.بالإضافة إلى تكتلها الفريد فيما يتعلق بأجناس أخرى مثل Bacillus و Xanthobacter. أظهرت شجرة النشوء والتطور لتسلسل الرنا الريبوسومي 16 S أن الأنواع المحددة من الآزوتوباكتر وغيرها من الأصناف المثبتة للنيتروجين مثل

زانثوباكتر، وسودوموناس، وكلبسيلا، لها روابط تطورية وثيقة. وأظهرت النتائج أيضًا أن تحسين تثبيت النيتروجين وخصائص تبادل الكاتيون كانت مؤشرات على زيادة نشاط الآزوتوباكتر في التربة المزروعة. يشير انتشار الآزوتوباكتر في التربة المزروعة إلى قدرتها على التكيف في الأراضي الزراعية. وخلصت الدراسة إلى أن ممارسات الزراعة تؤثر بشكل كبير على تجمعات الآزوتوباكتر، مما يفضل الأنواع ذات الكفاءة العالية في تثبيت النيتروجين. يمكن أن يؤدي تعزيز نشاط الآزوتوباكتر في التربة إلى تحسين توفر النيتروجين وخصوبة التربة. يوصى بتبني تناوب المحاصيل والتعديلات العضوية وتقليل استخدام الأسمدة الكيماوية لدعم تجمعات الآزوتوباكتر والحفاظ على صحة التربة. وبالتالي، يُنصح المزارعين والباحثين في منطقة كردستان في أربيل باستخدام الأسمدة الحيوية القائمة على الآزوتوباكتر على التربة الجيرية لزيادة خصوبة التربة والنشاط الميكروبي.

INTRODUCTION

The basis of sustainable agriculture is soil fertility, which supports both environmental health and global food security. The high calcium carbonate (CaCO3) content and alkaline pH of calcareous soils, like those found in the Kurdistan Governorate, pose serious obstacles to agricultural productivity, especially in arid and semi-arid areas (Yadav et al., 2021). These soils normally have low microbial diversity, poor structure, and poor nutrient availability, all of which adversely affect plant growth and productivity (Datsko et al., 2024).

Biological nitrogen fixation (BNF) by free-living nitrogen-fixing bacteria such as Azotobacter spp. has attracted enormous attention as one of the effective solutions necessary to overcome these problems. The natural process for BNF is vital in preserving soil fertility because symbiotic and free-living microbes transform atmospheric nitrogen in a form for plant use (de Lima et al., 2024). Azotobacter spp. are distinctive among these microbes because of their excellent capability in generating plant growth-promoting substances, fixing nitrogen in aerobic conditions, and improving soil health. According to Jnawali et al. (2015) and Gothandapani et al. (2017), these bacteria are vital for promoting environmentally friendly farming, reducing dependency on chemical fertilizers, and inhibiting contamination in the environment.

In addition to fixing nitrogen in soil, Azotobacter produces important phytohormones for example cytokinins, indole-3-acetic acid, and gibberellins that promote root and shoot development and plant productivity in general (Aasfar et al., 2021). They also play a part in biofertilizers because of their capacity to produce vitamins and siderophores, which defend plants against diseases (Yilihamu et al., 2020). Other research indicate that crops inoculated with Azotobacter exhibit improved growth parameters, such as increased leaf area, chlorophyll content, root biomass, and total yield (Moeinnamini et al., 2024). The possibility of combining biofertilizers based on Azotobacter with less chemical fertilizers to increase crop yields and soil health is reported by recent studies (Shahwar et al., 2023; Kaur et al., 2024). Azotobacter species have also tolerant to many agroclimatic zones because of their role and withstanding harsh environmental conditions, such as drought and low nutrient availability (Tripathi et al., 2024).

However, reports have recently showed that the farmers have challenges with cultivation of different cereal crops due to continuous effect of calcareous soil affecting microbial activities (Guo et al., 2019; Dou et al., 2024; Kasiviswanathan et al., 2024). This study hypothesized that abundance and activity of Azotobacter spp. in soil could be affected by different cultivation. Therefore, the objective of this study is to identify and quantify Azotobacter spp. in cultivated and uncultivated soils across different locations and analyse their phylogenetic relationships based on partial 16S rRNA gene sequences. The findings aim to guide the development of sustainable agricultural strategies that identify the role of microbial benefits while reducing external input dependence, thereby enhancing soil health and crop yield.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected on April 26, 2024, from various agricultural fields in different locations (Figure 1), including both cultivated and uncultivated lands in the Erbil governorate. The samples were taken from a depth of 30 cm to ensure consistency and relevance to root-zone analysis. These fields, managed by local farmers, were planted with a diverse range of crops, including barley, wheat, cucumber, potato, zucchini, bean, chickpeas, onion, tomatoes, and okra. The study aimed to analyze the characteristics of the soil across multiple crop types and farming practices, offering insights into the impact of cultivation and crop diversity on soil health and nutrient composition. A total of 10 different fields (Table 1) were sampled for representativeness of the agricultural lands.

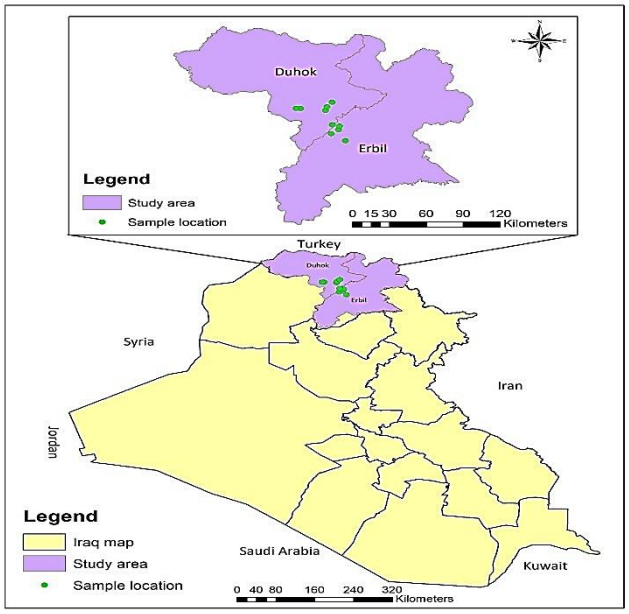

Figure 1. The map of soil sampling locations

Table 1. Soil sample locations of studied area

Locations	Reading (G.P.S)readings	
Gazna	36.280152	43.934864
Kawwrgosk	36.348541	23.803002
Shaxolan	36.388242	43.869998
mamandan	36.422907	43.877886
Darbistan al Ulya	36.575402	43.749604
Grdmamk	36.436127	43.813073
Sahaikh khanuk bag	36.609964	43.762044
Grdasen	36.655528	43.808648
Klkiy	36.590971	43.521488
Tall Jumal	36.592683	43.480764

Bacteria isolation

A total of 62 bacterial strains were successfully isolated from 12 subcultures using petri plate culture media. The nutrient agar media were used for the culture (Atlas, 2010), with composition including peptone, beef extract, and agar, provides essential nutrients and a stable surface for bacterial colonies to grow and for clear observation. The process involved careful preparation and inoculation of the culture media, followed by incubation under controlled conditions to promote bacterial growth. Each subculture was derived from specific environmental or experimental sources, ensuring a diverse representation of bacterial types. The isolates were distinct in morphology and growth patterns, highlighting the microbial diversity present in the samples. This systematic isolation process provides a foundation for further characterization, identification, and potential application of the bacterial strains in scientific and industrial research.

DNA extraction

Genomic DNA was extracted from 62 bacterial colonies isolated from the 10 different soil samples collected. The DNA isolation process was performed using the PGA Bacterial DNA Extraction Kit (PF230-050), manufactured in Iran. This method provided high-quality DNA suitable for downstream applications. The isolates were selected based on distinct colony morphology, ensuring diversity across the samples for molecular characterization.

The partial 16S ribosomal RNA (16S rRNA) gene was amplified using the Polymerase Chain Reaction (PCR) method. The 50 µl reaction mixture included 2x Taq DNA Polymerase Master Mix (AMPLIQON A/S), 10 pmol each of the forward primer (UN-16S: AGAGTTTGATCCTGGCTCAG) and reverse primer (UN-16S: GGCTACCTTGTTACGACTT), DNase-free water, and template DNA. The PCR program, performed on a Bioresearch PTC-200 Gradient thermocycler, consisted of the initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 35 seconds, primer annealing at 58°C for 35 seconds, extension at 72°C for 1 minute, and a final extension step at 72°C for 10 minutes. The amplification products were verified using a standardized protocol outlined in the reagent composition Table 2.

Table 2. 16S rRNA PCR Amplification Reagents

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	20 Pmol	3
3	Reverse Primer	20 Pmol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
Total			**50**

The DNA fragments were visualized using agarose gel electrophoresis after amplification. A 1.5% agarose gel that was prepared with 1X TBA buffer and stained with ethidium bromide, was run for 30 minutes under electric field. Subsequently, the DNA bands were examined under a UV trans-illuminator to confirm successful amplification of the 16S rRNA gene. The clear visualization of bands indicated accurate amplification.

Ten PCR products were sequenced using the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at the Microgene Center in Korea. Chromatograms of the sequences were edited and validated using Finch TV software. The 16S rRNA gene sequences obtained were analyzed using the Basic Local Alignment Search Tool (BLAST) available on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). This alignment method compared the laboratory-generated sequences with other biological sequences in the database to identify similarities and potential matches, providing insights into the phylogenetic relationships and taxonomy of the bacterial isolates.

Phylogenetic inferences

The phylogenetic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei

model in MEGA11 software, using partial 16S rRNA gene sequences. The 16S rRNA gene is widely used in bacterial phylogenetics and taxonomy to identify and determine evolutionary relationships among bacterial species.

RESULTS AND DISCUSSION

RESULT

Soil Physicochemical Properties

Table 3 displays results of physical properties of the studied soils. The results showed that the particle size distribution, textural classification, and bulk density of soil samples differed between different locations. The Silty loam (SiL), which was prevalent in Gazna, Kawrgosk, Shaxolan, and Darbistanaln Ulya soils, has a high silt concentration, moderate sand, and low clay contents. It provides superior drainage and nutrient-holding ability for crop farming. The maximum clay concentration of the silty clay loam (SiCL) found in Mamandan showed high in water retention. Grdmamk's loam (L) has the improved ratio of sand, silt, and clay, might be suitable for plant growth. Shaikh Khanuk Bag and Klkiy contain silty clay (SiC) rich nutrients and retains water, while having potential preventing root penetration and aeration. The relatively heavy clay loam (CL) of Grdasen showed well balances of water retention and drainage.

The bulk density (1.28 to 1.62 mg·m^{-3}) showed how soil compaction and porosity that differ from other areas, which has enormous influence on soil functionality. The higher porosity of Gazna (1.28 mg·m^{-3}) indicated better root penetration characteristics, water flow, aeration, and lower bulk density. Higher bulk density, as noted in Klkiy (1.62 mg·m^{-3}), on the other hand, denotes more compacted soils with less pore space. This might hinder root growth and limit water infiltration.

Table 3. Physical properties of the studied soils

Location	Particle Size Distribution mg.kg^{-1}			Textural Name	Bulk Density mg.m^{-3}
	Sand	Silt	Clay		
Gazna	440.50	542.00	53.20	SiL	1.28
Kawrgosk	127.70	674.30	199.00	SiL	1.38
Shaxolan	299.90	646.40	123.70	SiL	1.51
Mamandan	63.5	548.4	388.1	SiCL	1.33
DarbistanalnUlya	176.2	396.3	427.5	SiL	1.51
Grdmamk	389.0	375.6	235.4	L	1.34
Shaikh Khanuk bag	42.0	441.6	516.4	SiC	1.58
Grdasen	221.1	413.6	365.3	CL	1.32
Klkiy	424.2	103.4	472.4	SiC	1.62
Tall Jumal	0.00	0.00	0.00	SiL	0.00

Table 4 displays the results of pH, EC, soluble cations, and anions of studied soils. The organic matter (OM) content varies from 10.42 to 31.6 g·kg^{-1}, indicating variability in soil fertility. Darbistanaln Ulya (31.6 g·kg^{-1}) and Klkiy (24.56 g·kg^{-1}) had higher OM, improving nutrient availability and water retention. However, Grdasen (10.42 g·kg^{-1}) had the lowest OM, potentially limiting fertility. The soils were slightly alkaline, with pH varying from 7.4 to 8.2, typical for calcareous soils (Table 4). This pH levels were generally suitable for microbial activity, specifically for most bacteria and some fungi, which flourish in neutral to slightly alkaline conditions. The pH was also suitable for most crops but may reduce the availability of some micronutrients, such as iron and zinc. Total $CaCO_3$ content varies, with Grdasen had the highest concentration (590 mg·kg^{-1}) that could cause poor drainage and nutrient imbalances in the soil. Active $CaCO_3$ levels were relatively moderate across locations but higher in Gazna and Shaxolan, which might affect soil structure and plant root interactions, and potentially influencing water retention and aeration.CEC results range from 17.3 to 41.14 Cmol·kg^{-1}, indicating the capacity of the soils to retain and exchange essential nutrients (Table 4). Shaikh Khanuk Bag and Grdasen soils exhibited high CEC, indicating a greater ability to store nutrients, which could be beneficial for sustained plant growth. Lower CEC in Shaxolan might limit nutrient availability. Moreover, the EC results of the soluble cations and anions varied, varying from 0.402 to 1.98 dS ·m^{-1}, indicating non-saline soils. Klkiy showed the highest EC (1.98 dS m^{-1}), which is approaching the salinity threshold for sensitive crops and might potentially decrease yields if salinity increases. Higher concentrations of calcium (Ca^{2+}) and bicarbonate (HCO_3^-) were noted in many locations. For instance, Darbistanaln Ulya has notably high levels of Ca^{2+} (18.71 mmole·L^{-1}), which might improve soil structure but could cause calcium-induced nutrient imbalances.

PCR amplification of partial 16S rRNA gene

Figure 2 illustrates the results of the chromatogram sequencing of the 16S rRNA partial gene for the nitrogen-fixing bacterium Azotobacter spp. Phylogenetic analysis and molecular identification depend on this sequencing data. The chromatogram showed low baseline noise or overlapping peaks for each nucleotide (A, T, G, and C) throughout the sequence. This showed excellent sequencing results, implying precise base calling and reliable data for further examination. The consistent sequencing performance was supported by regions with uniform peak heights. The 16S rRNA gene was reliable target for identified Azotobacter spp. because it is largely conserved across bacterial species. According to the result, the identified Azotobacter spp. was a nitrogen-fixing bacteria that lives freely, which was essential in improving soil fertility and promoting plant growth.

Table 4. Results of pH, EC, Soluble Cations, and Anions of Studied Soils

Location	Organic Matter $gm.kg^{-1}$	pH	Total $CaCo_3$ $mg.kg^{-1}$	Active $(CaCO_3)$ $gm.kg^{-1}$	CEC $Cmol.kg^{-1}$	EC $dS.m^{-1}$	Soluble cation and anions ($mmole.L^{-1}$)							
							Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	HCO^{-3}	CO^{-3}	CL^{-}	$SO4^{-2}$
Gazna	13.8	7.6	340	50	20.0	0.549	3.35	0.90	0.85	0.61	4.80	N.D.	1.8	1.68
Kawrgosk	13.7	7.5	260	28	34.59	0.864	2.75	1.25	1.13	1.45	4.60	N.D.	2.2	1.39
Shaxolan	19.3	7.4	170	44	17.30	0.674	2.50	1.00	0.71	0.37	3.90	N.D.	1.2	1.49
Mamandan	19.8	7.6	390	9.35	21.37	0.632	0.57	0.48	0.21	0.04	0.25	N.D.	0.61	0.43
Darbistanaln Ulya	31.6	7.5	210	20.13	22.28	0.480	18.71	1.23	1.24	0.17	3.26	N.D.	1.4	1.34
Grdmamk	16.5	8.2	270	38	19.68	0.608	2.3	0.80	0.81	0.72	3.75	N.D.	1.6	1.19
Shaikh Khanuk bag	21.3	8.2	300	8.48	41.14	0.740	0.31	0.25	0.16	0.02	0.04	N.D.	0.42	0.21
Grdasen	10.42	7.4	590	10.20	40.43	0.402	0.00	0.00	0.00	0.00	0.00	N.D.	0.00	0.00
Klkiy	24.56	7.5	234	10.36	31.90	1.98	0.79	0.61	0.39	0.01	0.45	N.D.	0.91	0.53
Tall Jumal	22.8	8.2	255	7.12	38.01	0.740	0.23	0.20	0.14	0.02	0.04	N.D.	0.42	0.67

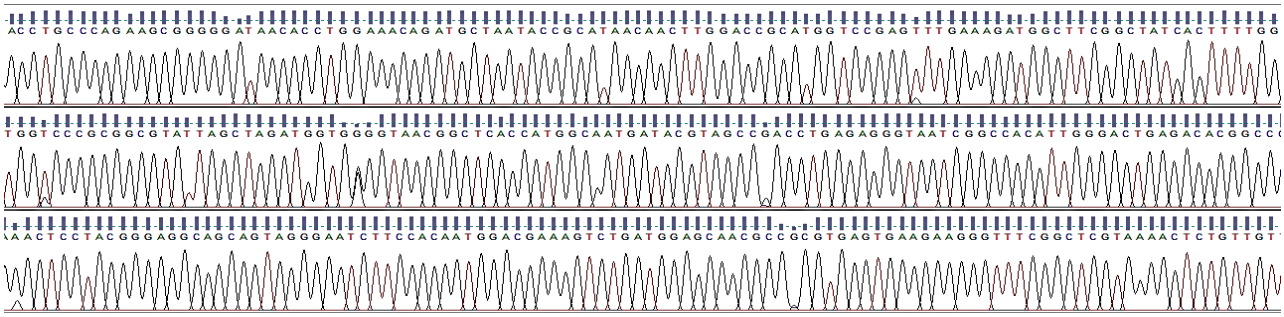

Figure 2. The chromatogram sequences result of partial gene of 16s rRNA sequences of Azotobacter spp.

Molecular Identification of Genus and Species of Bacteria

Table 5 shows the results of identified genus and species of bacteria. The table lists the bacterial species isolated from cultivated and uncultivated soils across different locations and crops. The bacterial genera identified primarily belong to the nitrogen-fixing and beneficial soil bacterial groups, including Azotobacter, Xanthobacter, Azospirillum, and Klebsiella, as well as other such as Staphylococcus, Pseudomonas, and Vibrio.

The most common species found in both cultivated and uncultivated soils belonged to the genus Azotobacter, specifically Azotobacter chroococcum (Table 5). This dominance emphasizes its significance as a nitrogen-fixing bacterium that improves soil fertility by transforming atmospheric nitrogen into forms that plants can use. Other identified Azotobacter spp. including A. beijerinckii, A. vinelandii, A. salinestris, and A. tropicalis, suggested that this genus is widely distributed over a range of soil conditions. Crop type, soil type, and OM content, which might probably have impact on this diversity.

The species diversity of cultivated soils was higher than that of uncultivated soils (Table 5). For example, some beneficial genera, such as Azotobacter, Xanthobacter, Azospirillum, and Klebsiella, were found in cultivated soils. These microorganisms support plant growth, inhibit pathogens, and aid in the cycling of nutrients in the soil. Although Azotobacter spp. were still common in uncultivated soils, fewer genera were found.

Table 5. Identified Genus and Species of Bacteria

Isolation sources	Locations	Bacterial Identified	Accession Numbers
Gazna	Cultivated Barley	*Azotobacter chroococcum*	PQ218983
		Enterobacter spp.	PQ218982
		Xanthobacter spp.	PQ218981
Gazna	Uncultivated	*Azotobacter beijerinckii*	PQ219610
		Azotobacter chroococcum	PQ219611
		Azospirillum spp.	PQ219612
		Klebsiella spp.	PQ219613
Kawrgosk	Cultivated Wheat	*Azotobacter chroococcum*	PQ219616
		Azotobacter beijerinckii	PQ219614
		Azospirillum sp.	PQ219617
Kawrgosk	Uncultivated	*Azotobacter chroococcum*	PQ276891
		Bacillus sp.	PQ276892
		Xanthobacter sp.	PQ276893
Shaxolan	Cultivated Cucumber	*Vibrio sp.*	PQ219929
		Staphylococcus sp.	PQ219930
		Azotobacter beijerinckii	PQ219931
Shaxolan	Uncultivated	*Azotobacter chroococcum*	PQ220249
		Azotobacter beijerinckii	PQ220250
		Xanthobacter sp.	PQ220251
Mamandan	Cultivated Potato	*Azotobacter vinelandii*	PQ249673
		Azotobacter beijerinckii	PQ249675
		Xanthobacter sp.	PQ249676

		Azotobacter chroococcum	PQ249674
Mamandan	Uncultivated	*Azotobacter vinelandii*	PQ249678
		Azotobacter chroococcum	PQ249679
		Azotobacter beijerinckii	PQ249680
		Bacillus sp.	PQ249681
		Staphylococcus sp.	PQ249682
DarbistanalnUlya	Cultivated Zucchini	*Azotobacter vinelandii*	PQ256802
		Azotobacte chroococcum	PQ256803
DarbistanalnUlya	Uncultivated	*Azotobacter chroococcum*	Seq1
		Xanthobacter sp.	Seq2
Grdmamk	Cultivated Bean	*Azotobacter chroococcum*	PQ276672
		Pseudomonas japonica	PQ276675
		Azospirillum sp.	PQ276673
		Klebsiella sp.	PQ276674
Grdmamk	Uncultivated	*Azotobacter vinelandii*	PQ276676
		Azotobacter chroococcum	PQ276677
		Azotobacter beijerinckii	PQ276678
		Xanthobacter sp.	*PQ276679*
Shaikh Khanuk bag	Cultivated Chickpeas	*Azotobacter chroococcum*	PQ276680
		Azotobacter vinelandii	PQ276681
		Staphylococcus sp.	PQ276682
		Azospirillum sp.	PQ276683
Shaikh Khanuk bag	Uncultivated	*Azotobacter salinestris*	PQ276684
		Azotobacter chroococcum	PQ276685
		Xanthobacter sp.	PQ276686
		Enterobacter sp.	PQ276687
Grdasen	Cultivated Onion	*Azotobacter chroococcum*	PQ276688
		Azotobacter beijerinckii	PQ276690
		Azotobacter bryophylli	PQ276689
Grdasen	Uncultivated	*Azotobacter beijerinckii*	PQ276694
		Azotobacter chroococcum	PQ276691
		Azotobacter salinestris	PQ276692
		Azotobacter vinelandii	PQ276693
		Azotobacter bryophylli	PQ276695
Klkiy	Cultivated Tomatoes	*Azotobacter chroococcum*	PQ299180
		<Azotobacter vinelandii	PQ299181
		Azotobacter nigricans	PQ299182
		Azotobacter armeniacus	PQ299183
		Azotobacter bryophylli	PQ299184
		Azotobacter tropicalis	PQ299185
		Azotobacter beijerinckii	PQ299186
Klkiy	Uncultivated	*Azotobacter chroococcum*	PQ299187
		Azotobacter vinelandii	PQ299188
		Azotobacter nigricans	PQ299189
		Azotobacter armeniacus	PQ299190
		Azotobacter salinestris	Q299191
		Azotobacter tropicalis	PQ299192
Tall Jumal	Cultivated Okra	*Azotobacter vinelandii*	PQ276894
		Azotobacter beijerinckii	PQ276895

		Vibrio sp.	PQ276896
		Staphylococcus sp.	PQ276897
Tall Jumal	Uncultivated	*Azotobacter chroococcum*	PQ220249
		Azotobacter beijerinckii	PQ220250
		Xanthobacter sp.	PQ220251

Phylogenetic inferences

Figure 3 displays the result of phylogenic tree of the soil bacterial species. The tree revealed discrete clusters that correspond to many bacterial taxa, such as Azotobacter, Xanthobacter Pseudomonas, Bacillus, Staphylococcus, and Klebsiella. These clusters had both genetic divergence between genera and genetic closeness within genera. The Azotobacter sp. cluster together as an example of their close evolutionary relationships and shared functions as nitrogen-fixing bacteria. Despite their occasional co-occurrence in soil ecosystems, Bacillus and Staphylococcus are members of distinct clades, indicating distinct evolutionary paths.

Species such as A. chroococcum, A. beijerinckii, A. vinelandii, and others form subclades within the genus, indicating the great diversity of Azotobacter. Their adaptability in different soil conditions and their roles in nitrogen fixing are revealed by this diversity. Bacillus and Xanthobacter spp. also showed genetic variation within their genera, indicating environmental or functional role differentiation. The evolutionary distances shown by the tree's branch lengths are shorter for closer relationships and longer for greater divergence. Azotobacter chroococcum was found in several sequences that were near to one another, indicating that isolates differed. In comparison to other Bacillus spp., Bacillus pumilus and Bacillus safensis have comparatively lengthy branches, suggesting more genetic divergence.

DISCUSSION

The study found that the alkaline pH between 7.4 and 8.2 and high CaCO3 content of calcareous soils in the studied regions were characterized by poor microbial activity and nutrient availability. CEC and OM differed between cultivated and uncultivated soils. The increased OM content of farm soils improved nutrient availability and microbial activity. This result is in agreement with a finding reported by Singh et al. (2018), which showed that higher microbial habitats and carbon availability perform better in soils richer with OM. In line with this, Rashid et al. (2019) reported that microbial inoculants in calcareous soils help to enhance CEC and reduce pH stress. Various soils and crops contains different microbial abundance depending on the soil physical and chemical characteristics of the soil (Panhwar et al., 2024).

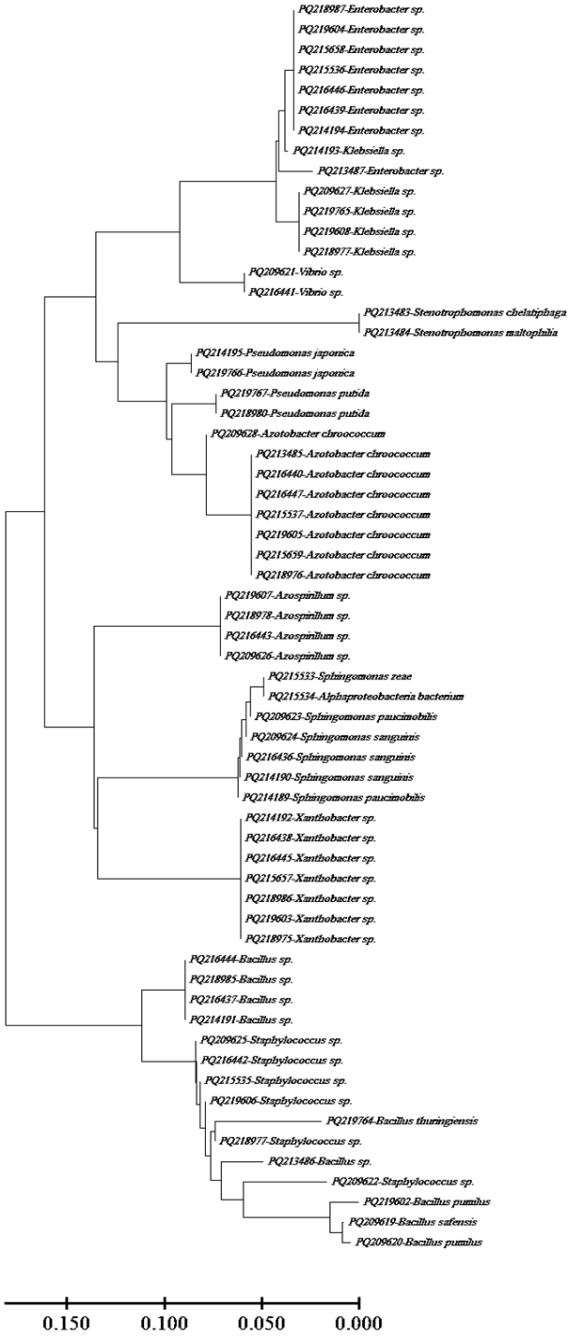

Figure 3. Phylogenic tree of the soil bacterial species. It was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software depend of –partial DNA sequences of 16S rRNA gene.

There were notable differences between cultivated and uncultivated soils for the identified Azotobacter species,

including A. chroococcum, A. vinelandii, A. beijerinckii, A. salinestris, and A. nigricans, with cultivated soils in general displaying a greater diversity. Root exudates and improved nitrogen cycles in the farmed environments that promote bacterial growth are responsible for this. Mahmood et al. (2020) reported that soil management practices have impact on the variety of nitrogen-fixing bacteria, including Azotobacter. In a similar manner, Yadav et al. (2021) found that through root-microbe interactions, cultivation encourages the growth of advantageous bacteria.

According to the 16S rRNA sequencing phylogenetic tree, the identified Azotobacter species and other nitrogen-fixing taxa such as Xanthobacter, Pseudomonas, and Klebsiella, have close evolutionary links. The high degree of similarity amongst these bacteria indicated that they have functional characteristics, such as nitrogen fixation and synthesis, which are advantageous for soil fertility and plant growth. According to Glick (2012), phylogenetic clustering is a sign of functional redundancy among soil microorganisms, which increases soil resistance in nutrient-deficient environments.

The findings showed that improved nitrogen fixation and cation exchange characteristics are indications of increased Azotobacter activity in the cultivated soils. Despite the limitations of high pH and CaCO3, cultivation probably promotes Azotobacter growth through improved aeration, organic supplements, and root exudation. Zaidi et al. (2017) obtained close results, in which Azotobacter inoculation increases the amount of OM and nitrogen in the soil, reducing the inherent negative effect of calcareous soils.

CONCLUSION

The study revealed that cultivation practices significantly impact the diversity, quantity, and activity of bacterial strains specially Azotobacter spp. in calcareous soils. It suggested that soil management plays a key role in improving soil fertility and crop productivity. Cultivated soils showed higher Azotobacter diversity and abundance due to improved OM content, nutrient availability, and aeration. Azotobacter spp. displayed increased activity in cultivated soils, contributing to better soil nutrients cycling and fertility. The study also highlights the importance of microbial diversity in sustainable agriculture, emphasizing the integration of microbial diversity, quantity, and activity for better soil health and reduced chemical dependence. Optimizing cultivation practices, including organic amendments and crop rotations, can enhance Azotobacter functionality, improving soil fertility, particularly in nutrient-poor calcareous soils. Farmers and researchers in the Kurdistan area of Erbil are advised to use biofertilizers based on Azotobacter on calcareous soils to increase soil fertility and microbial activity. It is also recommended that farmers use sustainable agricultural practices, such as the use of OM amendments and adopt crop rotation, to encourage the growth of microorganisms and soil health. It is also recommended that further study be conducted to assess any cereal crop and growing practices on Azotobacter activity in different agricultural soils.

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