

Thermotolerant and Halotolerant Endophytic Bacteria from Karbala, Iraq: Promising Biocontrol Agents against *Xanthomonas gardneri*

Zahida Hatif Mahdi¹, Bilal Saad Jalil², Namaa Sadeq Mohammed Ali³

¹ Department of Pathology Analysis, College of Applied Medical Sciences University of Kerbala, Kerbala, Iraq. Email: zahida.h@uokerbala.edu.iq.

² Department of Basic Sciences, College of Nursing, University of Kerbala, Kerbala, Iraq. Email: bilal.s@uokerbala.edu.iq.

³ Department of Basic Sciences, College of Nursing, University of Kerbala, Kerbala, Iraq. Email: namaa.s@uokerbala.edu.iq.

Email: bilal.s@uokerbala.edu.iq (Corresponding Author)



Received: 11 / 1 /2026

Accepted: 2 / 2 /2026

Published: 31 / 3 /2026

DOI:

10.65682/kjnhs.v2.i1.84-94

Abstract

Tomato is Significant for Iraq's food security and agricultural economy. Primarily serving regional and national markets, the Al-Sharia agricultural area in Karbala Governorate serves as a major production hub. Most especially bacterial spot brought on by *Xanthomonas gardneri*, phytosanitary problems always threaten the viability of this sector. This pathogen causes extreme defoliation and fruit damage, which results in major financial losses. Certain unique environmental conditions make disease control in Iraq more challenging. The region is defined by calcareous soils and summer temperatures typically exceeding 40°C. These conditions not only help the pathogen to spread through irrigation but also make some of the commercially available biocontrol products from temperate regions less effective. These products usually have trouble surviving in such challenging environments. The reliance on copper-based bactericides has raised concerns about environmental pollution and the development of resistant pathogen strains. Consequently, biological control using endophytic bacteria looks to be a wise choice. This study aimed to find local endophytic bacteria fit for these circumstances and determine their capacity to ward off other bacteria. Sixty bacterial isolates were obtained; seventeen different endophytes selected for characterization were discovered. Koch's postulates supported confirmation of the local *X. gardneri* isolate's pathogenicity. On the basis of phenotypic and biochemical characteristics, the promising isolates were distributed among the *Bacillus* and *Pseudomonas* genera. Significantly, isolates KSH-04 and KSH-07 (*Pseudomonas* sp.) were quite resilient; they grew at 5% NaCl concentrations and 42°C. In vitro studies revealed significant antagonism against *X. gardneri* linked to siderophore production. Greenhouse studies showed that, in comparison to the control, pre-treating with these stress-tolerant isolates reduced disease severity by more than 50%. According to this study, selecting ecologically friendly strains is vital as it offers a sustainable means of tomato production in dry and salty agroecosystem .

Keywords: stress tolerance, salinity, bacterial spot, *Xanthomonas gardneri*, endophytic bacteria, biocontrol.



1. Introduction

Tomato is a staple in diets and a major source of income for farmers; it is probably one of the most important vegetable crops in the world. Agriculture contributes significantly to Iraq's gross domestic product; in the southern and central regions, particularly tomato growing is quite significant. Famous for its rich soils and abundant vegetable production, the Al-Sharia area of the Karbala Governorate is a key source of fresh produce for the Iraqi market. But numerous biotic stresses are continuously threatening this productivity. Among them, *Xanthomonas gardneri*-related bacterial spot stands out as a terrible illness that severely compromises yield and fruit quality (Al-Rubaie & Al-Falooji, 2023). The illness manifests itself as soggy spots on the stems, fruits, and leaves, which soon develop into necrosis. Severe defoliation exposes fruits to sunburn, which lowers commercially acceptable yield (S. Sharma & Bhattarai, 2019) in really bad situations.

Iraq's management of bacterial spots presents several difficulties. The hot and dry temperature that dominates and the irrigation methods that produce microclimates with high humidity provide the ideal epidemiological setting for the pathogen. Most traditional management techniques have relied on copper-based pesticides and antibiotics. Not yet certain if this line of thought is actually ecologically sound. Recent research indicate that Middle Eastern copper-resistant *Xanthomonas* species have increased drastically, rendering these chemical treatments ineffective (Mansoor *et al.*, 2025). Moreover, the environmental footprint of heavy metal buildup in soils and the possible health risks linked to pesticide residues have driven a change toward sustainable agriculture methods. Development of environmentally friendly substitutes resistant to the shifting climate is urgently needed (Yusuf *et al.*, 2025) since they are effective against pathogens and are otherwise resilient.

Biological control, which uses helpful bacteria to fight plant diseases, has become one of the most important things to do for sustainable agriculture. Particularly beneficial are endophytic bacteria, microbes living inside plant tissues free from damage. Unlike soil dwellers or epiphytic microorganisms, endophytes are shielded from changes in the environment and have the same ecological role as vascular pathogens, therefore they are perfect choices for disease control (Ali *et al.*, 2024). These bacteria use biocontrol strategies include ISR induction in the host plant, competition for nutrients and space, and manufacture of antimicrobial metabolites (such as siderophores, hydrogen cyanide, and antibiotics) (Eid *et al.*, 2021).

Even if several biocontrol solutions have been successful around the world, ecological knowledge is still a major limitation in the case of dry and semi-dry areas such as Iraq. Developed in temperate regions, several commercially available biopesticides do not reach adequate population densities in the demanding Iraqi conditions. The calcareous soils of Karbala have a high pH and salinity. During the summer, the temperature is always above 40°C (Shrivastava & Kumar, 2015). Often leading to fast population decrease, introduced strains' inability to withstand these abiotic stresses makes them useless in field settings. Therefore, the choice of biocontrol agents should give environmental adaptability top priority rather than only rely on a pathogen-centric strategy. Native microbiomes of plants growing in challenging conditions are proven to be organically rich in stress-tolerant strains (Eckstien *et al.*, 2024).

The premise guiding this investigation is that healthy tomato plants growing in the harsh conditions of Al-Sharia, Karbala, house a distinctive collection of endophytic bacteria with dual features: the ability to inhibit *X. gardneri* and the physiological plasticity to withstand high temperature and salinity. This newly developed functional screening technique is natural. This research places top priority on phenotypical and physiological features essential for performance and survival in the local environment, above taxonomic identification alone.

This study aimed to: (1) identify and phenotypically characterize endophytic bacteria from various Karbala tomato genotypes; (2) verify the identity and pathogenicity of the local *X. gardneri* population; (3) assess whether bacterial isolates could withstand abiotic challenges such salinity and

temperature; (4) determine whether they could grow plants and combat disease in the lab; and (5) see if the selected stress-tolerant isolates could reduce disease severity in a greenhouse. This study aims to bridge the gap between lab testing and field application by selecting people who are ecologically pre-adapted to the difficult conditions of Iraqi agriculture.

2. Materials and Methods

2.1. Study Area and Sampling Strategy We did this study during the time for farming in 2025. We took samples of plants from tomato fields that people were farming for money in the Al-Sharia district, which is also known as the Al-Sharia Agricultural Region in the Karbala Governorate, Iraq. The Al-Sharia district is, in a place that does not get a lot of rain it gets very hot in the summer. People use water to help their plants grow. We picked the samples in a way we made groups and then picked some from each group by chance. Ten different tomato genotypes (cultivars and hybrids) were selected, including the widely cultivated local cultivars 'Zubair ', 'Safwan', and 'Julnar', as well as commercial hybrids like 'Shorouq' and 'Majd'. Samples included roots, lower stems, leaves, and seeds collected from healthy, symptomless plants that were growing in proximity to diseased plants. This selective sampling was intentional to isolate endophytes associated with disease-suppressive capacity.

2.2. Endophytic Bacterial Isolation Sterile polyethylene bags carried samples to a private specialized lab in Baghdad for quick examination. To eliminate epiphytic bacteria, rigorous surface sterilization techniques were applied. To thoroughly cleanse tissues of any adhering dirt, tap water was used. Following a three-minute immersion in 2.5% sodium hypochlorite (NaOCl), samples were then bathed three times in sterile distilled water and immersed for one minute in 70% ethanol. To evaluate the effectiveness of the surface sterilizing, 100 μ L of the last rinse water was seeded on Nutrient Agar (NA) medium. No microbiological growth following incubation suggested successful sterilizing.

Sterile saline solution (0.85% NaCl) was used to aseptically macerate the surface-sterilized tissues in a sterile mortar and pestle. Following sequential dilution of the macerate (10^{-1} to 10^{-6}), 100 μ L portions of appropriate dilutions were spread-plated onto NA medium. Plates were let to incubate at $30 \pm 2^\circ\text{C}$ for 48 hours. Selected, streaked, and stored in 20% glycerol at -20°C for long-term preservation were different bacterial colonies differing in shape (color, texture, height, margin). Isolates were first given distinct codes from the region (Karbala-Sharia: KSH), then a number (such as KSH-01 to KSH-17).

2.3. Pathogen Verification and Isolation (Koch's Postulates) The pathogenic bacteria tested in this investigation were taken from tomato leaves showing classic signs of bacterial spot found on various Karbala fields.

Surface sterilised, ground in sterile water, and streaked onto Yeast Dextrose Calcium Carbonate (YDC) agar, a semi-selective medium for *Xanthomonas*, lesion borders were isolated. Selected were yellow, mucoid, convex colonies usual of the genus.

Pathogenicity Test: A spectrophotometer ($\text{OD}_{600} = 0.2$) was used to adjust a bacterial suspension of the separated pathogen to 1×10^8 CFU/mL in order to verify identity and virulence. Healthy tomato seedlings (cv. 'Local Karbala') were sprayed with the suspension until run-off. A negative control came from sterile water. For 48 hours, polyethylene bags were used to cover plants in order to keep a high level of moisture. Then, they were placed in a greenhouse with a temperature of $28 \pm 2^\circ\text{C}$. Confirmation of pathogenicity came from the development of water-soaked lesions progressing to necrosis in seven to ten days. Fulfilling Koch's postulates, the pathogen was recovered once again from these fresh lesions. The isolate was determined as *Xanthomonas gardneri* (Potnis *et al.*, 2015) depending on colony morphology on YDC and the particular hypersensitive reaction.

2.4. Phenotypic and biochemical identification of endophytes Standard bacteriological tests were used to distinguish the genus level among the isolates.

Following 24 hours of growth on NA, morphologically traits—colony color, texture, elevation, and margin—were documented.

Gram-positive and Gram-negative bacteria were distinguished using a typical gram staining process.

- Following 48 hours, isolates were streaked on King's B (KB) medium and examined under UV illumination ($\lambda=365$ nm). The presence of pyoverdine siderophores—a key trait for the genus *Pseudomonas*—is suggested by the creation of greenish-yellow fluorescent pigment.

Using 3% hydrogen peroxide, catalase activity was examined; oxidase reagent discs were used to gauge oxidase activity. On starch agar plates flooded with iodine solution, amylase—starch hydrolysis—was examined.

2.5. Abiotic Stress Tolerance Screening Isolates underwent stress tolerance assays to confirm appropriateness for the Iraqi habitat.

Temperature Tolerance: Isolates were put into 10 mL of Nutrient Broth (NB) and kept at various temperatures: 30°C (control), 37°C, 40°C, and 42°C. Growth was measured spectrophotometrically twenty four hours afterwards (OD600). Thermotolerant separates at 42°C with OD600 > 0.5 were thought of (Nwamaka Anthonia *et al.*, 2020) .

2.6. Plant Growth-Promoting Traits

- **Siderophore Production:** Qualitative assay using Chrome Azurol S (CAS) agar. Formation of an orange-yellow halo around the colony against a blue background indicated positive activity (Tafaraji *et al.*, 2025). The halo diameter was measured.
- **Phosphate Solubilization:** Tested on Pikovskaya's agar containing insoluble tricalcium phosphate. Clear halos around colonies indicated the solubilization of inorganic phosphate.

2.7. In Vitro Antagonism Assays The antagonistic activity of endophytic isolates was tested against the confirmed *X. gardneri* isolate using the agar diffusion method. The pathogen was grown in NB and adjusted to 1×10^8 CFU/mL. 100 μ L of the pathogen suspension was spread-plated on NA plates. Wells (6 mm diameter) were punched in the agar and filled with 50 μ L of endophytic bacterial suspension (adjusted to 1×10^8 CFU/mL). Plates were incubated at 28°C for 48-72 hours. The diameter of the inhibition zone (clear area around the well) was measured in millimeters. Each treatment was replicated three times (Thomas & Upreti, 2014)

2.8. Greenhouse Bioassays Tomato seedlings (cv. 'Zubair', susceptible to Xg) were grown in a sterilized peat moss/perlite mix (1:1 v/v) in a greenhouse with natural light and temperatures ranging 25-32°C. At the 4-5 leaf stage, seedlings were treated with bacterial suspensions (1×10^8 CFU/mL) of selected isolates by foliar spray until run-off and soil drenching (10 mL per plant). After 7 days, plants were challenge-inoculated with the *X. gardneri* suspension (1×10^8 CFU/mL). Control plants were treated with sterile water. Disease severity was assessed 21 days post-inoculation using a 0-5 rating scale where: 0 = no symptoms; 1 = 1-5% leaf area affected; 2 = 6-10%; 3 = 11-25%; 4 = 26-50%; 5 = >50% or dead plant. The experiment was arranged in a Randomized Complete Block Design (RCBD) with four replicates per treatment.

2.9. Statistical Analysis Data were analyzed using SPSS software version 26.0. Prior to analysis, data were tested for normality using the Shapiro-Wilk test. Homogeneity of variances was tested using Levene's test. Analysis of variance (ANOVA) was performed for all measured variables. Treatment means were separated using the Least Significant Difference (LSD) test at a probability level of $P < 0.05$. Results in tables are presented as mean \pm standard deviation (SD).

3. Results

3.1. Pathogen Verification The isolated pathogen produced typical yellow, mucoid, convex colonies on YDC agar. Inoculation onto tomato seedlings resulted in the development of water-soaked lesions that evolved into necrotic spots surrounded by chlorotic halos within 7 days. Re-isolation from these lesions yielded colonies identical to the original isolate. No symptoms were observed in the water control. These results fulfilled Koch's postulates, confirming the identity of the local isolate as *Xanthomonas gardneri*.

3.2. Phenotypic Characterization

A total of 60 isolates were recovered from the tissues that were cleaned on the surface.

After making these isolates pure and grouping them based on how they looked they kept 17 endophytic isolates for a closer look at the bacterial isolates.

The people doing this work wanted to learn more about the isolates they had found.

So they decided to take a look, at the 17 endophytic isolates they had kept.

- Group 1 (KSH-04, KSH-07, KSH-11): The bacteria in this group are Gram- rods. When we grow them on a food called NA they form big and creamy colonies. What is really cool about these bacteria is that when we shine a light on them called UV light on a special medium called King's B they make a greenish-yellow color that glows. This tells us that they are a type of bacteria called pseudomonads. We also did some tests on them. Found out that they have something called oxidase and catalase. Because of all these things we learned about them we think they belong to the *Pseudomonas* sp group. The KSH-04 and KSH-07 bacteria are really good at making this yellow color, even more, than the others.

- Group 2 (KSH-09, KSH-12): These isolates were Gram-positive rods, capable of forming endospores. Colonies were rough and white to cream. They were positive for catalase and amylase but negative for oxidase. These were tentatively assigned to the genus *Bacillus* sp.

- Group 3 (Others): The other isolates were less common and had different traits; many had weak growth or did not show any antagonism in the first tests.

3.3. Abiotic Stress Tolerance The abiotic stress tolerance screen clearly distinguished the found genera (Table 1). Resilience was shown to be higher in *Pseudomonas* sp. isolates KSH-04 and KSH-07. They demonstrated vigorous growth at the control temperature (30°C) and maintained substantial growth even at 42°C (OD600 > 0.8). Similarly, these isolates tolerated high salinity levels, growing well in media containing 5% NaCl. In contrast, the *Bacillus* isolates (KSH-09, KSH-12) showed moderate growth at 40°C but failed to grow at 42°C and were significantly inhibited at 5% NaCl. This confirms that the *Pseudomonas* sp. isolates are physiologically adapted to the extreme conditions of the study region.

Table 1. Abiotic stress tolerance profile of endophytic isolates (Growth measured by OD600 after 24h).

Isolate Code	Tentative Genus	Growth at 30°C (Control)	Growth at 42°C	Growth in 5% NaCl
KSH-04	<i>Pseudomonas</i> sp.	1.25 ± 0.05	0.85 ± 0.04	0.78 ± 0.03
KSH-07	<i>Pseudomonas</i> sp.	1.22 ± 0.06	0.82 ± 0.05	0.75 ± 0.04
KSH-09	<i>Bacillus</i> sp.	1.10 ± 0.04	0.00 ± 0.00	0.42 ± 0.02
KSH-12	<i>Bacillus</i> sp.	1.08 ± 0.05	0.00 ± 0.00	0.40 ± 0.03

Values represent mean ± SD of three replicates.

3.4. Plant Growth-Promoting Traits Functional characterization demonstrated that the stress-tolerant isolates also possessed beneficial traits (Table 2). KSH-04 and KSH-07 produced large halos on CAS agar (22.0 mm and 19.5 mm respectively), indicating potent siderophore production. They also solubilized phosphate effectively, forming clear zones on Pikovskaya's agar. The *Bacillus* isolates produced smaller siderophore halos and KSH-09 lacked phosphate solubilization ability.

Table 2. Functional traits of selected endophytic isolates.

Isolate Code	Genus	Fluorescence on KB	Siderophore Halo (mm)	Phosphate Solubilization Halo (mm)
KSH-04	<i>Pseudomonas</i> sp.	+	22.0 ± 1.2	15.0 ± 0.8
KSH-07	<i>Pseudomonas</i> sp.	+	19.5 ± 1.0	12.5 ± 0.9
KSH-09	<i>Bacillus</i> sp.	-	11.0 ± 0.8	0.0 ± 0.0
KSH-12	<i>Bacillus</i> sp.	-	9.5 ± 0.7	6.0 ± 0.5

3.5. In Vitro Antagonism The antagonistic potential of the isolates against *X. gardneri* is presented in Table 3. All four selected isolates significantly inhibited the growth of the pathogen compared to the control. The *Pseudomonas* sp. isolates were significantly more effective than the *Bacillus* isolates. KSH-04 produced the largest inhibition zone (18.5 mm), followed closely by KSH-07 (17.2 mm). The clear, distinct inhibition zones suggested the production of diffusible antimicrobial metabolites.

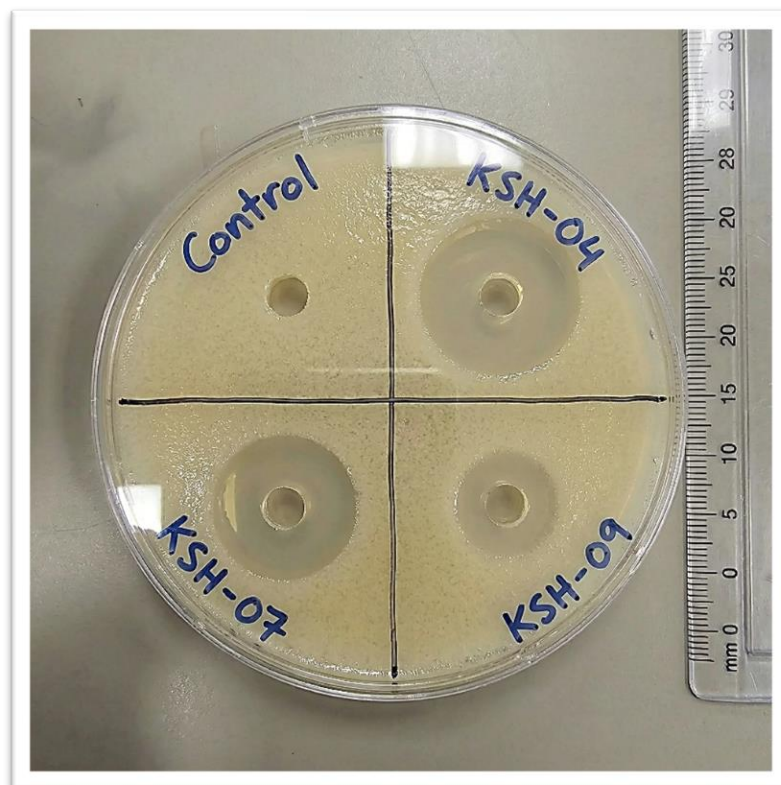


Figure 1. In vitro antagonistic activity of selected endophytic isolates against *Xanthomonas gardneri* using agar diffusion method. The plate was divided into four quadrants: Control (no inhibition), KSH-04 (18.5 mm), KSH-07 (17.2 mm), and KSH-09 (12.0 mm). Values represent mean inhibition zones from three independent replicates. Scale in mm.

Table 3. *In vitro* inhibitory activity against *Xanthomonas gardneri* (zone of inhibition in mm).

Isolate Code	Genus	Inhibition Zone Diameter (mm)*
KSH-04	<i>Pseudomonas</i> sp.	18.5 ± 0.6 a
KSH-07	<i>Pseudomonas</i> sp.	17.2 ± 0.5 a
KSH-09	<i>Bacillus</i> sp.	12.0 ± 0.8 b
KSH-12	<i>Bacillus</i> sp.	10.5 ± 0.7 b
Control	-	0.0 ± 0.0 c

Means (± SD) followed by the same letter do not differ significantly according to LSD test (P<0.05).

3.6. Greenhouse bioassays Table 4 reveals the effectiveness of the endophytes in regulating bacterial spot under greenhouse settings. The *in vitro* antagonism was reflected in the outcomes. Plants inoculated with *Pseudomonas* sp. isolates KSH-04 and KSH-07 had notably lower disease severity (1.8 and 2.0 on the 0-5 scale, respectively) than the pathogen control (4.2). This shows a disease decline of 52.4% and 57.1%. Moderate but statistically significant control was offered by the *Bacillus* isolates, which lowered severity to 2.7 and 2.9. Treated with KSH-04, plants stayed green with little spotting whereas control plants underwent extreme defoliation and necrosis.



Figure 2. Comparative severity of bacterial spot caused by *Xanthomonas gardneri* in tomato plants under greenhouse conditions. (A) Pathogen control showing severe necrotic lesions, chlorotic halos, and extensive tissue damage (disease index 4.2). (B) Plants pre-treated with *Pseudomonas* sp. KSH-04 exhibiting significantly reduced symptom development and preserved leaf integrity (disease index 1.8). Images captured under controlled greenhouse conditions using high-resolution macro photography.

Table 4. Endophyte efficiency on bacterial spot severity in greenhouse settings.

Treatment	Disease Severity (0-5 Scale)	Disease Reduction (%)
KSH-04 (<i>Pseudomonas</i> sp.)	1.8 ± 0.2 c	57.1
KSH-07 (<i>Pseudomonas</i> sp.)	2.0 ± 0.3 c	52.4
KSH-09 (<i>Bacillus</i> sp.)	2.7 ± 0.4 b	35.7
KSH-12 (<i>Bacillus</i> sp.)	2.9 ± 0.3 b	30.9
Pathogen Control	4.2 ± 0.4 a	0.0

Means (± SD) followed by the same letter do not differ significantly (LSD, P<0.05).

4. Discussion

Given the challenging conditions of Iraqi agriculture, the findings of this study highlight the need of selecting ecologically appropriate biocontrol agents. The successful isolation of *Pseudomonas* sp. strains KSH-04 and KSH-07, capable of tolerating temperatures up to 42 degrees Celsius and 5% NaCl, answers a significant gap in the application of biocontrol in semi-arid and dry environments (Kumar *et al.*, 2022). It is unusual to find strains with such a combination of stress tolerance even if *Pseudomonas* species are well recognized as effective biocontrol agents globally; this is a significant finding for regional agriculture (Etesami & Maheshwari, 2018). Challenging the popular belief that *Pseudomonas* strains are susceptible to high temperatures, our data suggest that the Karbala area acts as a natural selection force promoting the formation of strong microbial communities.

The antagonistic activity observed in vitro results from several processes together with subsequent disease control observed in greenhouse trials. King's B medium's strong fluorescence and CAS agar's large halos point to siderophore production, especially pyoverdines. Siderophores are important for iron competition because they take iron from the rhizosphere or apoplast and make it unavailable to the pathogen (Eid *et al.*, 2021; Gu *et al.*, 2020). Many microbial enzymes and virulence factors depend on iron; hence, limiting iron availability successfully reduces *X. gardneri*'s pathogenicity. The finding that the *Bacillus* isolates showed less antagonism and generated smaller siderophore halos helps the theory that iron chelation is the main means of suppression in this pathosystem.

Still, the over 50% decrease in disease severity under greenhouse conditions points to mechanisms other than basic competition being at work. Among the several secondary metabolites fluorescent pseudomonads make are phenazines, pyoluteorin, and hydrogen cyanide, all of which directly inhibit pathogens (Santoyo *et al.*, 2016). Recent research suggests that even though these metabolites were not measured in this study, they might have caused the antagonism that was seen. Moreover, endophytes' capacity to colonize internal tissues indicates they can eliminate the pathogen by niche occupation, a vital process for regulating vascular diseases like *Xanthomonas* (A. Sharma *et al.*, 2022).

A key point of this research was the emphasis on Induced Systemic Resistance (ISR). Well-known priming agents are endophytic bacteria. Before pathogen inoculation, KSH-04 and KSH-07 may have stimulated the inherent immune response of the plant. Since the biocontrol agent need not be found exactly at the site of infection, this systematic protection is especially important. (Yu *et al.*, 2022) showed that tomato plants can upregulate defense-related genes by *Pseudomonas* endophytes, which offers cross-protection against bacterial infections. Together, direct antagonism from siderophores and indirect defense from ISR probably explain why KSH-04 is so effective.

Perhaps the most important contribution of this work is its stress tolerance data. For the Iraqi summer, where soil surface temperatures might be even hotter, the capacity to develop at 42°C is absolutely essential. Strains lacking this thermotolerance would perish quickly upon application. Given the salinization problems in central Iraq, the salt tolerance (5% NaCl) is just as crucial. Stress tolerance and biocontrol effectiveness are correlated, so ecological fitness seems to be the most important factor for success in this place (Hashem *et al.*, 2019). The *Bacillus* isolates showed less thermotolerance than the *Pseudomonas* isolates in this particular setting, which might help to explain their somewhat inferior performance in the greenhouse experiment despite their spore-forming nature and typically considered robustness.

Further adding to the worth of these isolates are the functional properties of phosphate solubilization observed in KSH-04 and KSH-07. Important agricultural problems in the calcareous soils of Iraq are phosphorus fixation. Dual-benefit strains that can dissolve phosphate while inhibiting pathogens aid to meet sustainable intensification targets (Wahid *et al.*, 2020). Rather only as biopesticides, these isolates can therefore be created as biofertilizers to increase overall agricultural output.

Though this functional screening study did not primarily aim for molecular identification, the phenotypic and biochemical profiling provided a strong starting point for genus-level identification appropriate for applicants. Both species-level confirmation and investigation of the genetic basis of stress tolerance call for molecular instruments including 16S rRNA or *gyrB* sequencing (Masmoudi *et al.*, 2025). Finding particular stress-response genes (e.g., heat shock proteins, osmoprotectant synthesis routes) and antibiotic biosynthetic clusters will be the main emphasis of future research based on genomic analysis of KSH-04 and KSH-07. This will help to better understand their mechanism and direct design strategies.

5. Conclusions

This study successfully discovered indigenous endophytic strains *Pseudomonas* sp. KSH-04 and KSH-07 from tomato plants developing in Karbala, Iraq. High antagonistic activity against *Xanthomonas gardneri* combined with good resistance to strong salinity and high temperatures define these isolates. Under greenhouse settings, their capacity to drastically lower disease severity emphasizes their possible suitability as practical biocontrol agents adapted for the demanding agroecosystems of Iraq. Giving environmental competence first priority in the screening process, this research offers a blueprint for creating strong biocontrol solutions for dry and semi-arid areas all around the world.

6. References

- Ali, Md. A., Ahmed, T., Ibrahim, E., Rizwan, M., Chong, K. P., & Yong, J. W. H. (2024). A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. *Heliyon*, *10*(11), e31573. <https://doi.org/10.1016/j.heliyon.2024.e31573>
- Al-Rubaie, G. H. S., & Al-Falooji, S. A. A.-H. K. (2023). Chemical and Molecular Detection of New Strains of *Xanthomonas Campestris* pv. *Vesicatoria* and *Pseudomonas putida* Associated with Tomato Crop. *IOP Conference Series: Earth and Environmental Science*, *1262*(3), 032031. <https://doi.org/10.1088/1755-1315/1262/3/032031>
- Eckstien, D., Maximov, N., Margolis, N., & Raanan, H. (2024). Towards sustainable biocontrol: inhibition of soil borne fungi by microalgae from harsh environments. *Frontiers in Microbiology*, *15*. <https://doi.org/10.3389/fmicb.2024.1433765>
- Eid, A. M., Fouda, A., Abdel-Rahman, M. A., Salem, S. S., Elsaied, A., Oelmüller, R., Hijri, M., Bhowmik, A., Elkelish, A., & Hassan, S. E.-D. (2021). Harnessing Bacterial Endophytes for

- Promotion of Plant Growth and Biotechnological Applications: An Overview. *Plants*, 10(5), 935. <https://doi.org/10.3390/plants10050935>
- Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and Environmental Safety*, 156, 225–246. <https://doi.org/10.1016/j.ecoenv.2018.03.013>
- Gu, S., Wei, Z., Shao, Z., Friman, V.-P., Cao, K., Yang, T., Kramer, J., Wang, X., Li, M., Mei, X., Xu, Y., Shen, Q., Kümmerli, R., & Jousset, A. (2020). Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. *Nature Microbiology*, 5(8), 1002–1010. <https://doi.org/10.1038/s41564-020-0719-8>
- Hashem, A., Tabassum, B., & Fathi Abd Allah, E. (2019). *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi Journal of Biological Sciences*, 26(6), 1291–1297. <https://doi.org/10.1016/j.sjbs.2019.05.004>
- Mansoor, T., Ibrahim, Y. E., Al-Masrahi, A. A., Alhashel, A. F., & Al-Saleh, M. A. (2025). Copper Sensitivity, Host Resistance, and Bacteriophage Biocontrol of *Xanthomonas euvesicatoria* pv. *euvesicatoria*, the Causal Agent of Bacterial Spot in Pepper and Tomato Plants in the Riyadh Region, Saudi Arabia. *HortScience*, 60(8), 1313–1318. <https://doi.org/10.21273/HORTSCI18663-25>
- Masmoudi, F., Al Naimi, L., Trigui, M., Al Safran, M., Tounsi, S., & Saadaoui, I. (2025). Novel Thermo-Halotolerant Bacteria *Bacillus cabrialesii* Native to Qatar Desert: Enhancing Seedlings' Growth, Halotolerance, and Antifungal Defense in Tomato. *Journal of Plant Growth Regulation*, 44(2), 587–604. <https://doi.org/10.1007/s00344-024-11460-2>
- Nwamaka Anthonia, D., Edna Ifeoma, C., & Charles Onuora, C. (2020). Evaluation of Different Salts and Heavy Metal Concentrations on Bacterial Biofilm from Selected Surface and Borehole Water Samples. *Frontiers in Environmental Microbiology*, 6(2), 11. <https://doi.org/10.11648/j.fem.20200602.11>
- Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E., & Jones, J. B. (2015). Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology*, 16(9), 907–920. <https://doi.org/10.1111/mpp.12244>
- Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, Ma., & Glick, B. R. (2016). Plant growth-promoting bacterial endophytes. *Microbiological Research*, 183, 92–99. <https://doi.org/10.1016/j.micres.2015.11.008>
- Sharma, A., Kaushik, N., Sharma, A., Marzouk, T., & Djébal, N. (2022). Exploring the potential of endophytes and their metabolites for bio-control activity. *3 Biotech*, 12(10), 277. <https://doi.org/10.1007/s13205-022-03321-0>
- Sharma, S., & Bhattarai, K. (2019). Progress in Developing Bacterial Spot Resistance in Tomato. *Agronomy*, 9(1), 26. <https://doi.org/10.3390/agronomy9010026>
- Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, 22(2), 123–131. <https://doi.org/10.1016/j.sjbs.2014.12.001>
- Tafaraji, S. H., Abtahi, S. A., Jafarinia, M., & Ebadi, M. (2025). Screening, molecular identification, and evaluation the effects of indigenous Plant Growth-Promoting Rhizobacteria on growth indices and nutrient uptake of chamomile (*Matricaria chamomilla*) under saline conditions. *Frontiers in Microbiology*, 16. <https://doi.org/10.3389/fmicb.2025.1551310>

- Thomas, P., & Upreti, R. (2014). Testing of Bacterial Endophytes from Non-Host Sources as Potential Antagonistic Agents against Tomato Wilt Pathogen <i>Ralstonia solanacearum</i>; *Advances in Microbiology*, 04(10), 656–666. <https://doi.org/10.4236/aim.2014.410071>
- Wahid, F., Fahad, S., Danish, S., Adnan, M., Yue, Z., Saud, S., Siddiqui, M. H., Brtnicky, M., Hammerschmidt, T., & Datta, R. (2020). Sustainable Management with Mycorrhizae and Phosphate Solubilizing Bacteria for Enhanced Phosphorus Uptake in Calcareous Soils. *Agriculture*, 10(8), 334. <https://doi.org/10.3390/agriculture10080334>
- Yu, Y., Gui, Y., Li, Z., Jiang, C., Guo, J., & Niu, D. (2022). Induced Systemic Resistance for Improving Plant Immunity by Beneficial Microbes. *Plants*, 11(3), 386. <https://doi.org/10.3390/plants11030386>
- Yusuf, A. G., Al-Yahya, F. A., Saleh, A. A., & Abdel-Ghany, A. M. (2025). Optimizing greenhouse microclimate for plant pathology: challenges and cooling solutions for pathogen control in arid regions. *Frontiers in Plant Science*, 16. <https://doi.org/10.3389/fpls.2025.1492760>