Bacterial Endophytes from *Hibiscus sabdariffa* Roots: A Source of Growth-Promoting Abilities Candidates

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Abstract

This study aimed to isolate and characterize native strains from the endophytic rhizospheric of roselle (*Hibiscus sabdariffa* L.) plants to evaluate their potential as plant growth-promoting rhizobacteria (PGPRs). A total of 32 bacterial isolates were obtained and subjected to various biochemical tests, as well as assessments of plant growth-promoting traits such as phosphate and potassium solubilization, indole acetic production, nitrogen fixation assay and evaluating its capacity to endure varying temperatures as a measure of stress. The results showed that the inner shell of roselle plants roots in Aswan hosts a diverse group of microbes, as HER18Disolates were able to produce the highest value of indole among 32 isolates, reaching (38.87 mgl^{-1}), while isolate HER9D excelled in nitrogen fixation, reaching (43.87mgl^{-1}). HER35D and HER13D were the highest in the (PSI) and (KSI), respectively (2.54 and 3.63 $cm)^{-1}$. It is interesting to note that most of the isolates excelled in tolerating high temperatures such as HER9D, HER20D, HER37D and HER25D. Some isolates also excelled in combining multiple functions of growth-promoting traits and stress resistance, such as HER18D.Based on PGP traits, different isolates were identified that are considered potent and can be used and applied as PGPRs for different plants and improving organic agriculture to achieve a sustainable environment.

Keywords: Endophytic; Rhizobacteria; PGPRs; Heat tolerance; Medicinal plants microbiome

1. Introduction

The prerequisite for novel and secure bioactive formulations is still escalating to achieve health safety, agricultural sustainability, and relief in every aspect of human life. To combat various new emerging diseases as a result of modern lifestyle and deteriorating environmental health, researchers are forced to investigate novel and potential bioactive constituents **[1]**. Increasing crop yield, which frequently depends on the use of chemical fertilizers, is necessary to feed the growing population in emerging nations. However long-term use of these fertilizers has been demonstrated to reduce soil bacterial diversity **[2],** and may have negative environmental repercussions, including increased soil and groundwater contamination **[3]** and phosphorus and nitrogen leakage into groundwater **[4]**. Utilizing effective, nutrient-mobilizing microorganisms to lessen reliance on chemical fertilizers is one method to improve the sustainability of agricultural practices **[5, 6]**.

Medicinal plants contain phytocompounds, which are essential for maintaining health. Additionally, they provide a habitat for a range of endophytic bacteria **[7].** The majority of endophytes are microorganisms that reside in plant tissue without causing harm to the host plant.

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Certain endophytes stimulate plant growth by giving them the nutrients and resources they require and by regulating plant growth **[8]**. Roots, stems, leaves, seeds, fruit, tubers, ovaries, and legume nodules have been observed to harbor endophytic bacteria **[9].** Through nitrogen fixation, phytohormone synthesis, and resistance to harmful microorganisms, the host plants may achieve a rise in growth **[10].** Furthermore, endophytic bacteria and rhizobacteria create secondary compounds on their host plants that influence the physiological growth of the plants and give them disease resistance **[11].** The endophytic bacteria may be able to get nutrients from plant metabolism and defend against environmental stressors due to this relationship. Thus, compared to rhizosphere bacteria, endophytic bacteria are better able to withstand biotic and abiotic stress **[9].**

These useful soil microbes, however plentiful in the rhizosphere, are generally under-exploited as bio-inoculants for upgrading crop production, particularly under abiotic stress **[12].** Roselle (*Hibiscus sabdariffa* L.) is a medicinal plant widely cultivated for its calyxes, which are rich in anthocyanins and antioxidants. Like many other medicinal and aromatic plants, roselle hosts a diverse microbiome, especially within its root system. Endophytic bacteria have gained attention due to their potential role in promoting plant growth via mechanisms known as plant growth-promoting rhizobacteria (PGP) traits **[13].**

These PGP traits include solubilization of essential minerals, phytohormone production, and nitrogen fixation. Many studies have confirmed that different types of such endophytic bacteria are capable of supporting plant growth and health by using them as a vital combination in processes including plant stimulation, biofertilization, and biological control **[14-16],** which reduces the use of harmful traditional methods and helps maintain agricultural sustainability.

This study aimed to isolate and characterize native strains from the endophytic rhizosphere of roselle plants to evaluate their potential as plant growth-promoting rhizobacteria.

2. Materials and Methods

2.1 Study area and sample collection

Roselle root samples were obtained from several sites at the Experimental Farm, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt (23°59'52"N 32°51'35"E, 170 m) (Figure 1).

Healthy and mature roselle plants were randomly selected, and undamaged young root samples were taken. These root samples were used as the source for isolation of endophytic bacteria. Sampling was conducted to recover a broad range of cultivable bacteria, hence enhancing the likelihood of isolating bacteria with useful features for biotechnological applications. The tightly adhering dirt to the root, regarded as rhizosphere soil, was collected and placed in a sterilized plastic bag for physico-chemical analyses (Table 1)**.** The physical and chemical analysis was conducted according to the protocols of **[17, 18]**.

All samples were enclosed in sterile zip-locked bags, transported to the laboratory in an icebox, and processed within 24 hours.

Figure 1: (A) Roselle plant (*Hibiscus sabdariffa*) at the sampling site. **(B)** Collection of root samples for isolation of endophytic bacteria.

Table.1: Soil profile: physical and chemical properties of the rhizosphere soil

Characteristics	Value
Physical properties (%)	
Clay	3.07
Silt	0.43
Sandy	96.50
Textural class	Sandy
Chemical properties	
pH (1:2.5 extract)	8.06
EC (dSm^{-1}) at 25°C	0.53
Soluble anions (meq/L)	
CO_3^{-2}	---
HCO_3^-	7.02
Cl^-	3.53
SO_4^{-2}	0.48
Soluble cations (meq/L)	
Ca^{++}	3.15
Mg^{++}	1.07
K^+	0.88
Na^+	0.84
Available macronutrients (ppm)	
Available N	131.0
Available P	10.0
Available K	180.0

2.2 Endophytic bacteria isolation

Roots were surface sterilized and processed for the isolation of endophytic bacteria using standard microbiological techniques **[19]** on nutrient agar and roselle medium with some minor modifications. Root samples were cleaned in a laminar airflow hood using running tap water and then distilled water. They were then immersed in 70% (v/v) ethanol for 30 seconds and 5% (v/v) sodium hypochlorite for three minutes. Lastly, sterile distilled water that had been autoclaved was used to wash them five times. The finished cleaned solution was spread out and cultivated on the medium **[20]** for 24 hours at 30°C to confirm surface sterilization.

To isolate endophytic bacteria from the sample, the roots were crushed in a sterile mortar and transferred to a 90 ml potassium chloride saline solution. The root explants were aseptically transferred and placed on two types of media nutrient and roselle agar. The cultured plates were incubated for 2–3 days at 30°C to allow endophytic bacteria to grow from the root explant **[21].**

Well-grown bacterial colonies which appeared from the root explant were randomly collected for further investigation. Pure cultures of each bacterial isolate were separately cultivated on nutrient broth (NB) (HiMedia, Mumbai, India) at 30°C and 150 rpm for 24 hrs. Liquid stock culture of each isolate in 20% (w/v) glycerol was prepared and kept at −80°C for the further studies.

2.3 Morphological and Microscopic Characterization

Each isolate was examined for colony morphology (color, shape, evaluation, margin, etc.) Gram staining, potassium hydroxide reaction and motility using light microscopy and standard staining methods.

2.4 PGPR Traits Assays

2.4.1 Indole Acetic Acid (IAA) production

Indole Acetic Acid was determined using the Salkowski reagent conducted protocol to estimate indole-related compounds **[22]**.

2.4.2 Phosphate and potassium solubilization ability

The bacterial endophytic isolates were screened for phosphate and potassium solubilization using Pikovskaya and Aleksandrov media. The media were inoculated with endophytic isolates and incubated for 7 days to assess the ability of entophytes to solubilize P and K consecutively **[23-25]**.

2.4.3 Nitrogen fixation assay

Nitrogen fixation assay was assessed on nitrogen-free semi-solid CCM medium **[26]** using the micro-Kjeldahl procedure method **[18].**

2.5 Physiological Behavior

2.5.1 Growth on CCM Medium

Growth efficiency was tested on N-deficient combined carbon sources medium to evaluate the ability of bacteria to grow in a nitrogen-free medium **[26]**.

2.5.2 Heat Tolerance Test

Each isolate was incubated at increasing temperatures up to 55°C to assess thermo-tolerance trait **[27, 28].**

2.6 Selection of the most potent endophytic bacterial

The strongest endophytic isolates were identified from among the 32 tested isolates that achieved positive results in PGP traits. The potent endophytic isolates were selected based on their specific effectiveness and proven efficacy in each trait.

2.7 Multifunctional potential of isolates

Thirty-two selected isolates from roselle were evaluated, and about 12 isolates were identified that were able to combine multiple characteristics of plant growth promotion and prove their efficiency in each characteristic and consider them as isolates that can be used to improve the growth of medicinal plants and crops in general.

3. Results

3.1 Morphological and Microscopic Growth of Bacteria Endophytic rhizobacteria

Morphological characterization revealed that 13 out of 32 isolates exhibit pinpoint, 10 moderate, 8 small and 1 big in size. In colony color were 15 white, 13 creamy white, 3 yellow, and 1 pink (Table 2). While, microscopic characterization in cell shape were 19cocci**,** 9 short rods, 3 long rods, and 1 oval. About 91% from isolates were Gram positive in contrast Gram negative recorded 9%. For motility test 23 isolates were motile however 9 isolates were non- motile.

Table 2: Morphological and microscopic characterization for 32 endophytic rhizobacteria isolates from roselle plants.

No.	Isolate Code	Colony morphology						Microscopic characterization			
		Size	Color	Margin	Form	Elevation	Opacity	Shape	Motility	Gram staining	KOH test
1	HER14B	Moderate	White	Entire	Circular	Flat	Opaque	Cocci	-	+	-
2	HER2$_w$D	Moderate	White	Entire	Circular	low convex	Opaque	Cocci	+	+	-
3	HER 4$_D$	Small	White	Entire	Circular	low convex	Opaque	Short rod	+	+	-
4	HER 10	Small	Creamy White	Entire	Circular	Flat	Opaque	Cocci	-	+	-
5	HER 12	Small	Creamy White	Entire	Circular	Flat	Opaque	Cocci	-	+	-
6	HER 24	Small	Creamy White	Undulate	Wrinkled	Flat	Opaque	Short rod	+	-	+
7	HER 28	Small	White	Entire	Circular	Flat	Opaque	long rod	+	+	-
8	HER 32	Moderate	White	Entire	Wrinkled	low convex	Opaque	oval	-	+	-
9	HER 47y	Pinpoint	Yellow	Entire	Circular	low convex	Opaque	Cocci	-	-	+
10	HER 35	Moderate	White	Entire	Circular	low convex	Opaque	Cocci	+	+	-
11	HER 17	Pinpoint	Creamy White	Entire	Circular	low convex	Opaque	Cocci	+	+	-
12	HER 13	Pinpoint	Creamy White	Entire	Circular	low convex	Opaque	Short rod	+	-	+
13	HER 1	Pinpoint	White	Entire	Circular	low convex	Opaque	Cocci	+	+	-
14	HER 2	Moderate	Creamy white	Irregular	Irregular	Flat	Opaque	Cocci	+	+	-
15	HER 3	Pinpoint	White	Entire	Circular	Flat	Semitransparent	Cocci	+	+	-
16	HER 4	Pinpoint	White	Entire	Circular	Flat	Semitransparent	Cocci	-	+	-
17	HER 5	Pinpoint	Creamy white	Entire	Circular	Flat	Opaque	Short rod	+	+	-
18	HER 16	Moderate	Creamy white	Entire	Circular	Flat	Opaque	Cocci irregular	+	+	-
19	HER 23	Moderate	Creamy white	Entire	Circular	low convex	Opaque	Short rod	+	+	-
20	HER 30	Pinpoint	White	Entire	Circular	low convex	Opaque	Cocci	+	+	-
21	HER 31	Pinpoint	Yellow	Entire	Circular	low convex	Semitransparent	Cocci	+	+	-
22	HER 42	Moderate	Creamy white	Entire	Circular	low convex	Opaque	Long rod	+	+	-
23	HER45	Pinpoint	White Pink	Entire	Circular	low convex	Opaque	Cocci	+	+	-
24	HER 41	Moderate	Creamy white	Entire	Circular	low convex	Opaque	Short rod	+	+	-
25	HER 20	Small	White	Entire	Circular	Flat	Opaque	Cocci	+	+	-
26	HER 25	Small	Creamy white	Entire	Circular	low convex	Opaque	Short rod	+	+	-
27	HER 37	Big	Creamy white	Entire	Irregular	low convex	Semitransparent	Cocci	-	+	-
28	HER 9	Pinpoint	White	Entire	Circular	Flat	Semitransparent	Cocci	+	+	-
29	HER 22	Pinpoint	White	Entire	Circular	low convex	Opaque	Short rod	-	+	-
30	HER 18	Moderate	White	Entire	Circular	low convex	Opaque	Long rod	+	+	-
31	HER 26	Small	Yellow	Entire	Circular	Flat	Semitransparent	Short rod	+	+	-
32	HER 19	Pinpoint	White	Entire	Circular	Flat	Opaque	Cocci	-	+	-

3.2 Ability of endophytic bacteria to produce indole-3-acetic acid (IAA)

The ability of endophytic bacteria to produce indole-3-acetic acid (IAA) was quantitatively evaluated using Salkowski's reagent. The concentration of IAA varied notably among the tested isolates, ranging from low to high levels (Figure 2).

The isolate HER31D recorded the highest IAA concentration (38.87 mgl^{-1}), followed by HE.R30D (35.74 mg L^{-1}) and HE.R37D (25.69 mg L^{-1}). These findings confirm the potential of certain isolates as strong phytohormone producers.

A representative image of the IAA colorimetric assay is shown in (Figure 3), where the intensity of the pink color corresponds to the amount of IAA produced, while the control tube remained colorless.

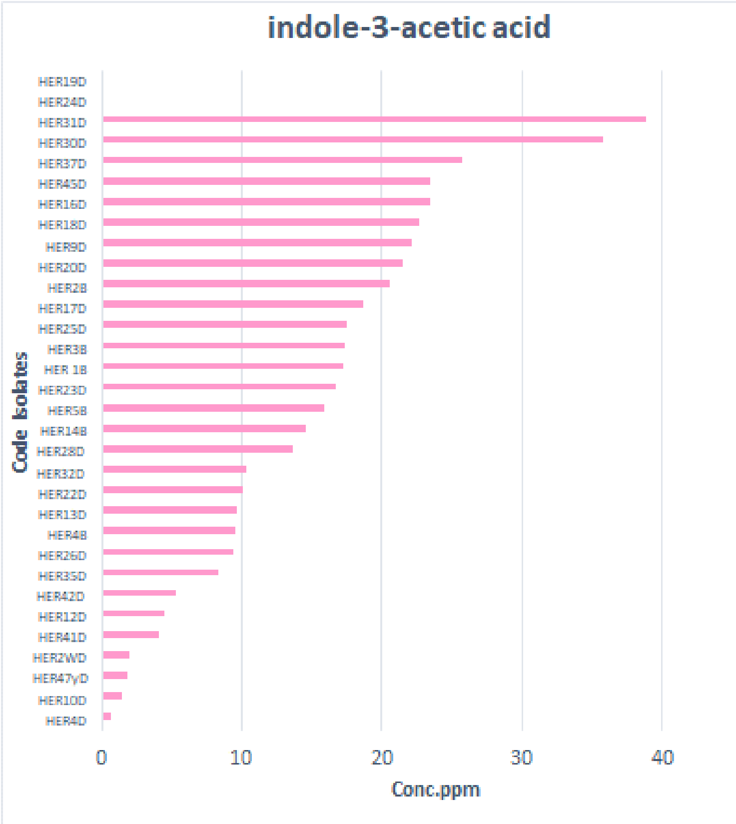


Figure 2: Quantitative estimation of IAA production (ppm) by the isolated endophytic bacteria.

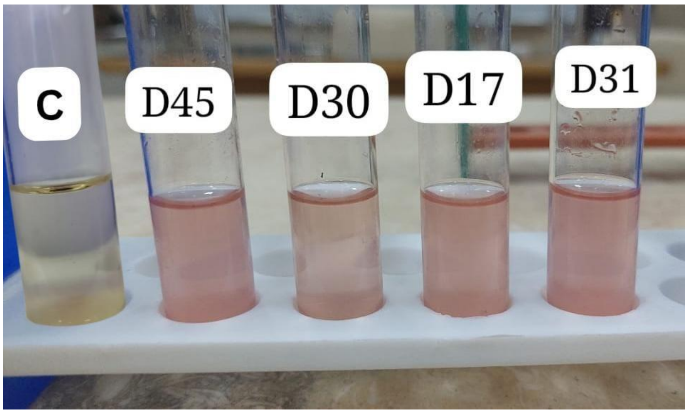


Figure 3: Indole-3-acetic acid (IAA) production test by selected bacterial isolates using Salkowski reagent. The development of a pink coloration indicates positive IAA production. (C: control without bacterial inoculation).

3.3 The endophytic bacteria of hibiscus have the capacity to dissolve potassium and phosphate.

Quantitative estimation of phosphate (Figure 4A) and potassium solubilization (Figure 4B) potential of the endophytic bacterial isolates were determined. The solubilization index (SI) was calculated on solid media using the halo zone diameter. Results showed variable capacities among isolates, with HER35D and HER17D exhibiting the highest phosphate solubilization index and HER13D and HER17D were showing superior potassium solubilization ability. Visual results of solubilization zones are shown in (Figure 4c, d, e).

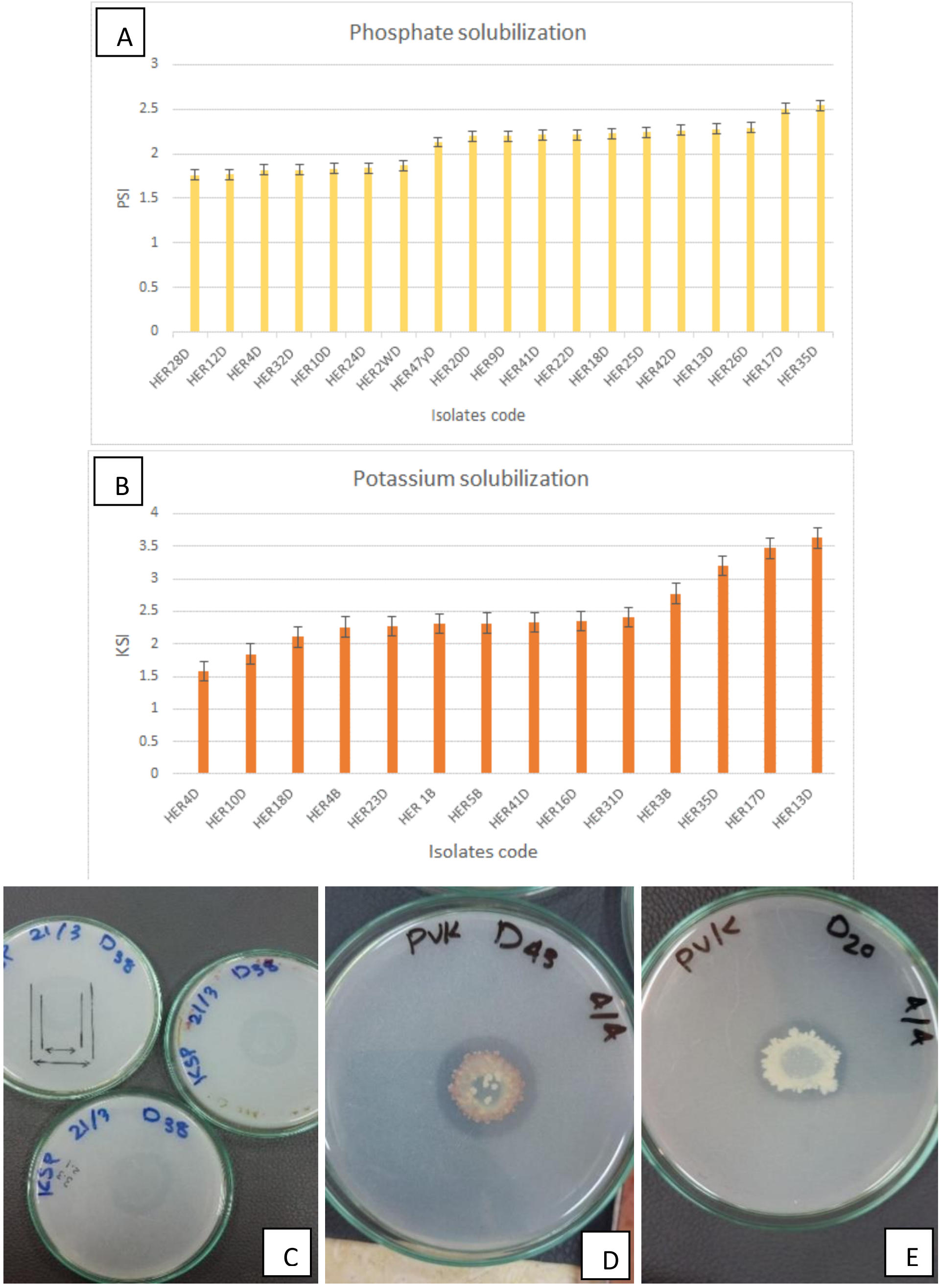
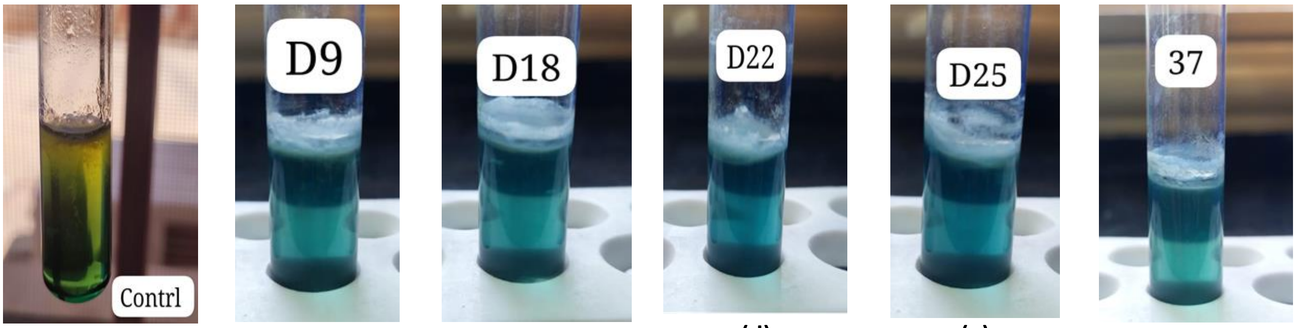


Figure 4: Representative plates and measurement schematic for phosphate and potassium solubilization: (A, B) Phosphate and potassium solubilization index by positive different isolates of endorhizospheric bacteria among 32 isolates; (C) Potassium solubilization on Aleksandrov medium with schematic marking of halo zone (outer diameter) and colony (inner diameter) used for solubilization index calculation; (D, E) Halo zone formation around the bacterial colony on PVK medium indicating phosphate solubilization.

3.4 The nitrogen fixation ability of endophytic bacteria harbored within the roots of roselle

Nitrogen fixation assay illustrates was evaluated by using CCM medium enhanced with Bromo thymol Blue (Figure 5). The medium's color changed visibly from green (control) to blue (for example isolates D9, D18, D22, D25, and 37which refers to isolates, HER9D, HER18D, HER22D, HER25D and HER37D), signifying nitrogenase activity and ammonia generation. To validate and quantify these observations, the fixed nitrogen in culture tubes was further determined using the Kjeldahl digestion method. This allowed accurate identification of the most efficient nitrogen-fixing among the 32 tested isolates. The combined qualitative and quantitative evaluation confirms the ability of several endophytes to biologically fix atmospheric nitrogen under in vitro conditions.



(a) (b) (c) (d) (e) (f)

Figure 5: Nitrogen fixation test on CCM medium. Control tube (a) showed no colour change, indicating no nitrogen fixation, (b–f) inoculated with isolates D9, D18, D22, D25, and 37, show blue coloration due to nitrogenase activity.

3.5 The ability of endophytic bacteria to grow under high temperature conditions

The thermo tolerance assay revealed notable variability among the 32 endophytic bacterial isolates in their ability to grow under elevated temperature conditions. As shown in (Table 3), all isolates demonstrated growth at 45°C, while the majority sustained moderate to strong growth at 50°C. However, only a subset (~50%) retained visible growth at 55°C after 48 hours of incubation. Notably, selected isolates exhibiting growth at higher temperatures are visually represented in (Figure 6), which illustrates colony development on nutrient agar plates incubated at 45°C, 50°C, and 55°C. These visual observations support the quantitative data, confirming the capacity of specific strains to tolerate thermal stress. Such thermo tolerant isolates (e.g., HER9d, $HER47_{\gamma}d$, HER18d, and HER20d) may represent promising candidates for the development of stress-resilient microbial inoculants, especially suitable for cultivation in hot climates and arid soils.

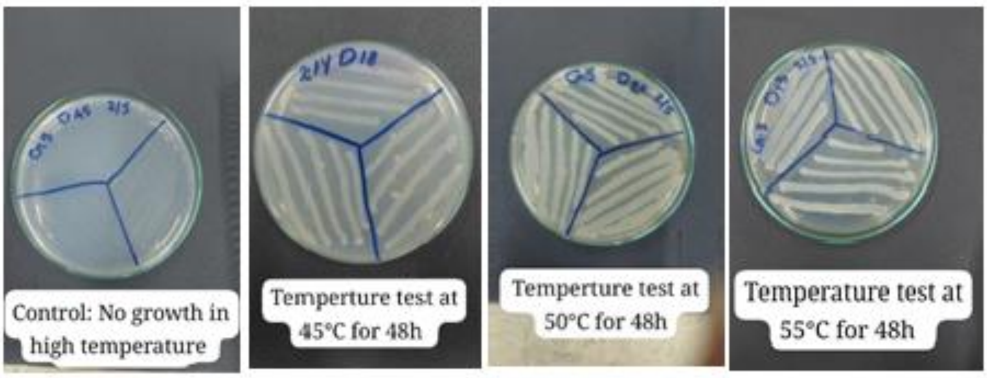


Figure 6: Temperature tolerance assay of endophytic bacterial isolates at 45°C (B), 50°C (C), and 55°C (D), for 48 hours.

Table 3: Thermo tolerance of Endophytic Bacterial Isolates at Different Temperatures.

Isolates Code	Temperature °C		
	45	50	55
HER14B	+	-	-
HER2wD	+++	++	++
HER4D	+++	++	+
HER10D	+++	++	+
HER12D	+++	++	+
HER24D	+++	+	+
HER28D	+++	++	++
HER32D	+++	++	++
HER47yD	+++	+++	+++
HER35D	+++	++	-
HER17D	++	+	-
HER13D	+++	+++	+++
HER1B	++	-	-
HER2B	++	++	-
HER3B	+++	+++	++
HER4B	+++	++	-
HER5B	-	-	-
HER16D	++	-	-
HER23D	+++	+++	-
HER30D	-	-	-
HER31D	++	-	-
HER42D	++	++	-
HER45D	+++	-	-
HER41D	+++	+++	+++
HER20D	+++	+++	+++
HER25D	+++	+++	+++
HER37D	+++	+++	+++
HER9D	+++	+++	+++
HER22D	+++	++	-
HER18D	+++	+++	+++
HER26	++	-	-
HER19D	+	-	-

- : No growth; + : weak growth; ++ : medium growth; +++ : high growth

3.6 Multifunctional Plant Growth-Promoting Endophytic self-Rhizobacteria (PGPR)

Figure (7) illustrates the multifunctional potential of the 32 tested endophytic bacterial isolates. Each isolate was evaluated for four key plant growth-promoting (PGPR) traits: only 6 isolates were superior in nitrogen fixation (mgl^{-1}), 19 isolates in phosphate solubilization index (PSI), 14 isolates in potassium solubilization index (KSI), and 30 isolates in indole-3-acetic acid (IAA) production, measured in ppm. The combination of these traits highlights the potential of certain isolates as multifunctional PGPR strains. The results showed that HER18D exhibited multifunctional PGP traits, including nitrogen fixation, phosphate and potassium solubilization, and IAA production, while HER22D, HER9D, and HER25D exhibited nitrogen fixation, phosphate solubilization, and IAA production.

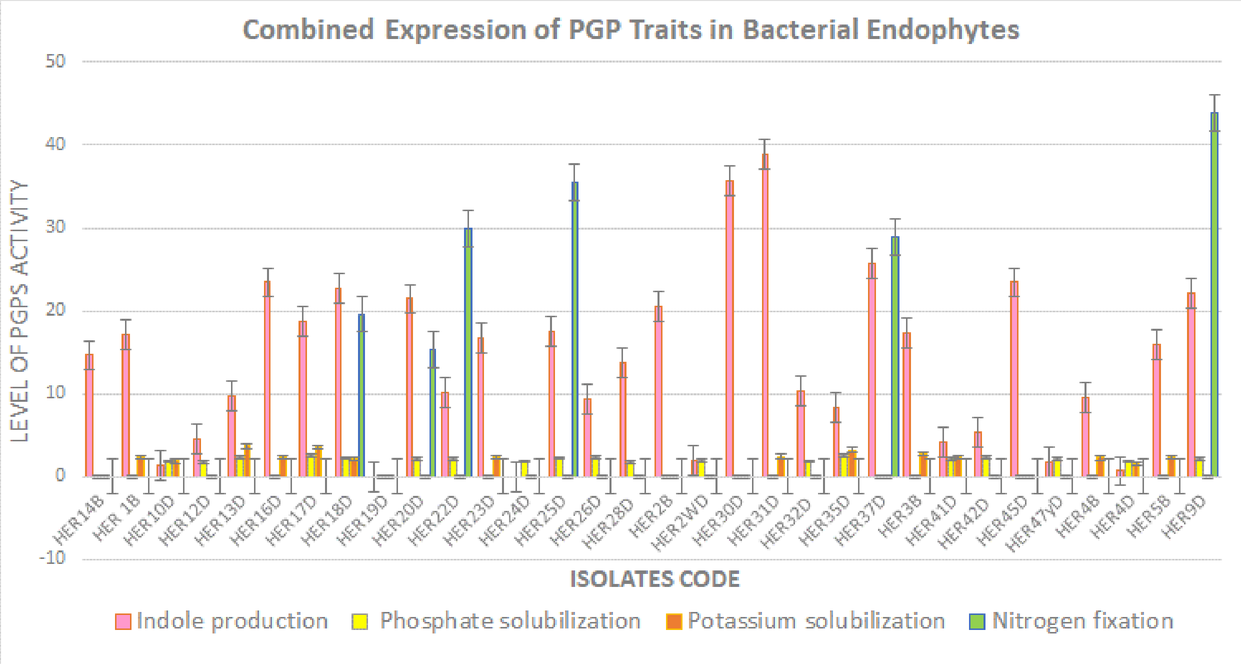


Figure 7: Multifunctional PGPR traits of endophytic bacterial isolates associated with *Hibiscus sabdariffa*.

3.7 Plant-promoting endophytic rhizobacteria isolated from roselle plant

Out of the original set of 32 endophytes, the 12 most effective bacterial isolates were chosen based on how well they performed in important PGPR features (Table 4). These include the synthesis of indole-3-acetic acid (IAA), the fixation of nitrogen, the solubilization of phosphate and potassium, and thermotolerance at 45°C, 50°C, and 55°C. Interestingly, a portion of these isolates showed multifunctionality, showing favorable outcomes for every investigated characteristic, which makes them viable options for additional use in stress-induced plant growth promotion.

Three of these isolates demonstrated a broad range of PGPR characteristics (HER18D, HER9D, HER22D, HER20D and HER25D) such as metabolic versatility and complete thermotolerance, suggesting that they could be used as multipurpose bioinoculants.

Table (4): The selected potent endophytes isolate recorded the highest values in PGP traits under *in vitro* conditions.

No.	Isolates code	IAA mg L^{-1}	N_2 assay mg L^{-1}	Solubilization		Temperature tolerance °C		
				P (PSI)*	K (KSI)**	45	50	55
1	HER35D	8.33	-	-	-	+++	++	-
2	HER17D	18.71	-	-	-	++	+	-
3	HER13D	9.65	-	-	-	+++	+++	+++
4	HER16D	23.41	-	-	-	++	-	-
5	HER30D	35.74	-	-	-	-	-	-
6	HER31D	38.87	-	-	-	++	-	-
7	HER20D	21.42	15.33	15.33	15.33	+++	+++	+++
8	HER25D	17.50	35.47	35.47	35.47	+++	+++	+++
9	HER37D	25.69	28.93	28.93	28.93	+++	+++	+++
10	HER9D	22.13	43.87	43.87	43.87	+++	+++	+++
11	HER22D	10.12	29.87	29.87	29.87	+++	++	-
12	HER18D	22.67	19.57	19.57	19.57	+++	+++	+++

- ; Not detected, *PSI; Phosphate Solubilization Index, **KSI; Potassium Solubilization Index

4. Discussion

Climate change poses a significant threat to medicinal plants and, by extension, both traditional and modern healthcare systems. In regions such as Aswan, characterized by a harsh desert climate, these impacts are particularly pronounced. Medicinal plants possess intrinsic antioxidant systems that enable them to adapt to a certain degree of environmental stress. However, under extreme conditions, the production of reactive oxygen species (ROS) increases, leading to oxidative damage. This includes disruption of chloroplast and mitochondrial functions, lipid peroxidation, impaired water uptake, and ultimately, compromised plant growth and development. The growing interest in medicinal plants due to their eco-friendly, non-toxic, and cost-effective nature, further underscores the need for effective strategies to ensure their resilience and sustainability **[29].**

The current study aligns with these concerns by isolating robust, multifunctional bacterial strains with plant growth promoting (PGP) traits that can enhance stress tolerance. These isolates display diverse physiological capabilities and adaptability, making them valuable for supporting plant health in challenging environments.

4.1 Endophytic bacteria and phytohormones

Given the limited information available on endophytes inhabiting the roots of *Hibiscus sabdariffa* L. (roselle), this study focused on isolating indole-rich bacterial endophytes from roselle roots and evaluating their potential for use in sustainable agriculture. A total of 32 isolates were recovered, several of which exhibited high levels of indole acetic acid (IAA) production. Specifically, isolates HER31D, HER30D, HER37D, and HER16D demonstrated IAA production levels of 38.87, 35.74, 25.69, and 23.41 mg L^{-1}, respectively.

These findings are consistent with previous reports on endophytic bacteria, such as *Lysinibacillus* and *Bacillus spp*. associated with *Prosopis strombulifera* **[30]**, *Gluconacetobacter diazotrophicus* in *Saccharum officinarum* **[31],** and similar associations in wheat **[32].** Such bacteria are known to produce IAA, thereby promoting plant growth and stress tolerance, especially in arid and semi-arid regions.

4.2 Phosphate and Potassium Solubilization by Endophytes

Phosphorus and potassium are essential macronutrients for plant development, and microbial solubilization of these nutrients plays a key role in sustainable soil fertility. In this study, 44% and 59% of the 32 isolates exhibited potassium and phosphorus solubilizing activity, respectively. Isolates HER13D, HER17D, and HER35D showed the highest potassium solubility indices (3.63, 3.47, and 3.20), while the same isolates also showed significant phosphate solubilization indices (2.28, 2.51, and 2.54).

These results are in agreement with **Hamza *et al*. [33]**, who reported P and K solubilization indices ranging from 1.0 to 5.5 in rhizospheric and endophytic bacteria. The dual solubilizing capacity observed in over half of the isolates is particularly noteworthy, highlighting their potential as biofertilizer components.

4.3 Nitrogen Fixation Activity

Nitrogen fixation is a critical microbial trait that contributes to sustainable agriculture by reducing dependency on chemical fertilizers. In nitrogen-free CCM media, six isolates demonstrated the capacity to fix atmospheric nitrogen. Quantitative estimation using the Kjeldahl method confirmed varied nitrogen fixation capacities among the isolates. Notably, HER9D, HER25D, and HER22D showed nitrogen fixation levels of 43.87, 35.47, and 29.87 mg L^{-1}, respectively, suggesting strong potential as biofertilizer candidates.

These findings are consistent with those of **Shi *et al*. [34],** who measured nitrogen fixation using the acetylene reduction assay (ARA), reporting activity as high as 67.45 nmol C_2H_4/mL.h. Despite methodological differences, both approaches affirm the significant nitrogen-fixing potential of endophytes isolated from medicinal plants.

4.4 Heat tolerance

Abiotic stresses such as heat and drought are major limitations to plant productivity **[35],** particularly in regions like Aswan with extreme climatic conditions. In this study, several isolates, including HER9D, HER18D, and HER25D, were able to tolerate temperatures up to 55°C. This thermotolerance is critical for microbial survival and efficacy under field conditions. Endogenous thermotolerant bacteria play a vital role in supporting host plant resilience, especially in arid soils. Over time, plants have adapted various mechanisms to overcome and tolerate adverse conditions, such as salinity, drought, heat, and other prevailing environmental factors. The use of PGP traits has emerged as a potential solution to this problem to enhance crop growth by producing a variety of bioactive compounds that improve plant tolerance to heat, drought, and nutrient deficiency **[36]**. These bacteria live within plant tissues (endophytes) and are well-adapted to high temperatures, allowing them to remain metabolically active under extreme environmental conditions. Their biochemical activities directly benefit the host plant in the following ways:

4.5 Enhanced Antioxidant Activity

Under heat and drought stress, plants generate reactive oxygen species (ROS) that damage cellular components. Thermotolerant bacteria help mitigate this by producing:

- **Enzymatic antioxidants** such as catalase, superoxide dismutase (SOD), and peroxidases.
- **Non-enzymatic antioxidants** like glutathione and phenolic compounds.

These reduce oxidative stress, stabilize cell membranes, and prevent damage to chloroplasts and mitochondria.

4.6 Stress-Induced Volatile Organic Compounds (VOCs)

Thermotolerant endophytes release VOCs that:

- Act as signaling molecules to trigger systemic resistance in plants.
- Induce expression of stress-responsive genes involved in drought and heat tolerance.

4.7 Production of Heat-Shock Proteins (HSPs) and Chaperones

Some endophytic bacteria induce or themselves produce **heat-shock proteins** that:

- Stabilize and refold denatured plant proteins.
- Protect photosynthetic machinery and maintain metabolic functions during thermal stress.

4.8 Induction of Systemic Tolerance (IST)

Through sustained colonization and signaling, thermotolerant bacteria can "prime" the plant to respond more quickly and robustly to future stress, a process known as induced systemic tolerance. This includes:

- Up-regulation of stress-responsive genes.
- Strengthening of root cell walls.
- Accumulation of defense-related enzymes and secondary metabolites.

Our findings are in line with **Saidan *et al*. [28],** who reported robust bacterial growth at 45–55°C in isolates from Jazan hot springs, where optimal conditions included temperatures between 45–50°C and a pH of 6.4. These results reinforce the value of isolating thermotolerant bacteria for use in high-temperature agricultural environments.

5. Conclusions

This study demonstrates the potential of endophytic bacteria isolated from roselle roots as plant growth-promoting agents with multifaceted functional traits. These include phytohormone production, phosphate and potassium solubilization, nitrogen fixation, and thermotolerance, all of which are crucial for enhancing plant resilience in arid and nutrient-poor soils.

Such isolates represent promising candidates for the development of environmentally friendly bioinoculants and sustainable agricultural biotechnologies. Future research should focus on molecular identification and comprehensive field trials to validate their effectiveness under real-world conditions.

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