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EXPLORING THE POTENTIAL OF ANTAGONISTIC ENDOPHYTIC BACTERIA ISOLATED FROM MEDICINAL PLANTS AND ITS SUPPRESSIVE EFFECT ON SOIL BORNE FUNGAL DISEASES OF TOMATO

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ABSTRACT: Medicinal plants host a variety of endophytic microbes that hold significant economic value. The present study is therefore focused on the isolation and identification of bacterial endophytes from medicinal plants growing in the arid region of Al-Arish (Egypt) and their potential role as bioinoculants for enhancing the growth of tomato plants. In this study, eight endophytic bacterial isolates showed direct broad spectrum *in vitro* antagonism against the tested fungi. On the basis of antagonism activity, these isolates were studied to identify based on their 16S rRNA gene sequences, as *Lysinibacillus fusiformis*, *Bacillus pumilus*, *B. siamensis*, *Paenibacillus peoriae*, *Paenib. Polymyxa*, *Pseudomonas aeruginos*a, *Brevundimonas diminuta*, and *Providencia vermicola*. The strains were screened for various plant growth-promoting (PGP) attributes, including indole-3-acetic acid (IAA), ammonia, siderophores, phosphatase, hydrolytic enzyme production and phosphate solubilization. Isolated bacterial strains have variable plant growth promoting activities. Two selected endophytic bacterial strains were assessed for their biological control potential against tomato fungal root rot disease caused by *Fusarium oxysporum* and *Rhizoctonia solani* to further evaluate their PGP abilities under greenhouse conditions. Under greenhouse, *B. pumilus* NAW4 and *P. aeruginos*a NAW6 proved effective in conferring positive benefits to tomatoes placed under stress as well as under normal growing conditions.

Key words: Medicinal plants, Endophytes, Antagonism activity, Bioinoculants, Tomato plants.

INTRODUCTION

The global population is expected to reach 9.2 billion by 2050, a rate of growth that is alarming. In order to keep up with the increase in food demand over the next 30 years, global agricultural production must increase by 60 to 70% above its present level (**De Silva *et al.*, 2019**). By increasing the yield potential of plants and protecting them from phytopathogens, it is possible to increase agricultural output. In the past, commercial fertilizers and pesticides were widely used to maximize agricultural production. Farmers are encouraged to use more eco-friendly alternatives to synthetic agrochemicals as a result of rising concerns about their harmful impacts on human and environmental health (**Solomon *et al.*, 2023**). In addition, excessive use of synthetic agrochemicals may result in the development of resistance in phytopathogens, necessitating the need for alternative approaches (**Hahn *et al.*, 2015**).

Utilizing biofertilizers and biopesticides as an alternative to synthetic chemical products for biocontrol and plant growth enhancement has become widespread. Symbiotic plant growth-promoting bacteria (PGPB) are essential for increasing plant yield and plant health in a wide range of environmental conditions (**Neyser *et al.*, 2016; Abdelaal *et al.*, 2015**). Endophytic microorganisms colonize various plant tissues and play a crucial role in plant growth and adaptation to biotic and abiotic stresses (**Ryan *et al.*, 2008; Vandana *et al.*, 2021**). These endophytic

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microorganism's ability to restrict the vascular growth of the target pathogen, hence reducing disease incidence and severity more efficiently (**de Lamo *et al.*, 2018; Constantin *et al.*, 2019**). They support plant growth through the formation of symbiotic partnerships, nitrogen fixation, phosphate solubilization, and the production of essential phytohormones (**Kandel *et al.*, 2017; Pinski *et al.*, 2019**). In the past decade, a large number of studies have supported the theory that plant growth-promoting bacteria (PGPB) enable plants to maintain productivity in the face of diverse biotic and abiotic stresses through a variety of mechanisms. The genera of these bacteria include *Rhizobium*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, *Pantoea*, *Achromobacter*, *Azospirillum*, *Burkholderia*, *Microbacterium*, *Methylobacterium*, and *Enterobacter* **(Grover *et al.*, 2011; Bharti *et al.*, 2013; Egamberdieva *et al.*, 2016; Li *et al.*, 2018*a*; Yadav and Meena, 2021**).

Reportedly, medicinal plants contain endophytes, which provide protection against infectious agents as well as the ability to adapt and persist in adverse environmental conditions. Therefore, it is essential to ascertain the endophytic diversity of medicinal plants (**Strobel, 2002**). Increasingly, it is recognized that the relationships between medicinal plants and specific bacterial endophytes can have a profound effect on the properties of the plants (**Ek-Ramos *et al.*, 2019**). These endophytes can produce the same secondary metabolites as the host plant (**Alvin *et al.*, 2014**). Historically, studies of medicinal plants have concentrated on their active compounds; but, more recently, researchers have begun to examine the microbiomes of these plants for their potential therapeutic use. Surprisingly, not only the plants themselves but also their accompanying microorganisms were capable of producing compounds with phytotherapeutic characteristics. Due to their unique and structurally distinct bioactive secondary compounds, medicinal plants have a distinct microbiome, which likely accounts for the high specificity of the associated microorganisms (**Qi *et al.*, 2012**).

The objective of the present work is to 1) isolate and identify novel antagonism bacterial endophytes associated with medicinal plants; 2) evaluate their roles in providing growth benefits using *in-vitro* assays; and 3) investigate the in-vivo growth promotion potential of the highly efficient strains of endophytic bacteria and biocontrol agent of soil borne pathogen on a tomato plant. Tomato (*Solanum lycopersicum* L.) plants were used as a model system to test the effects of these endophytes to decrease tomato root rot severity, caused by *Fusarium oxysporum* and *Rhizoctonia solani* in the greenhouse.

MATERIAL AND METHODS

Method for Sampling

Twenty samples of healthy-looking medicinal plants were collected from their natural habitats in Al-Arish City of Egypt. The samples of Whole plants, including root systems (10–30 cm depth) were aseptically harvested and shipped to the lab in sterile polyethylene bags with ice packs. If the samples needed to be stored for longer than 18 to 24 hours before processing, they were kept at 4°C.

Isolation of Endophytic Bacteria

The technique developed by **Aravind *et al.*, (2009)** was used to isolate endophytic bacteria from the root's interior tissues. The collected plants' roots were removed carefully soaked in water and then chopped into pieces that were 1–2 centimetres long. The roots were treated with 2% sodium hypochlorite for 10 minutes, followed by 1 minute of sterilization in 70% ethanol. The samples were then rinsed six times in distilled water to ensure sterility. The processed root samples were air-dried on sterile Whatman filter paper within a laminar air flow cabinet. The endophytic bacteria were cultured by shaking 10 grammes of roots in 10 millilitres of Tryptic Soy Broth medium at 180 revolutions per minute and at 28°C for four days. On Tryptic Soy Agar (TSA (BD, Difco Laboratories, Detroit, USA), serial dilutions of the root suspension (100 µl) were plated and then incubated at 28°C for two days. Following incubation, the most representative colonies were chosen and streaked twice on Nutrient agar (5.0 g/L of peptone; 5.0 g/L of sodium chloride; 1.5 g/L of meat extract; 1.5 g/L of yeast extract; 15 g/L of agar; pH 7.4±0.2) for purification. Various colony profiles were described based on morphological criteria (shape, colour,

elevation, diameter, and margin) (**Bergey *et al.*, 2010**). For future usage, the purified isolates were stored in the refrigerator.

In vitro Dual-Culture Assay

Using *Rhizoctonia solani*, *Fusarium oxysporum*, and *Altrnaria* sp., *in vitro* assays were conducted to determine whether or not certain endophytic bacteria could inhibit the growth of fungal pathogens. The phytopathogenic fungi were obtained from a stock of reference cultures held in the Laboratory of phytopathology, Agriculture Faculty, Suez Canal University. All preserved cultures of phytopathogenic fungi were transferred to potato dextrose agar (PDA) (20 g of glucose، 200 g of potato, 15 g of agar, 1000 mL of sterile water). plates and incubated at 28°C in the dark for seven days. The antifungal activity of all bacterial isolates was tested against phytopathogenic fungi on PDA plates using the protocol reported by examined **Skidmore and Dickinson (1976)**. Briefly, a mycelial plug of growing Fungus was deposited in the middle of the PDA medium, and endophytic bacteria were streaked 2 cm away from it on two sides. The plates were then incubated at 28°C for five days, or until the leading edge of fungus in the control group reached the plate's edge. Tests were performed in triplicate. The percentage of growth inhibition was computed using a method presented by **Vincent (1947)**.

$$Percentage\ of inhibition = \frac{C-T}{C} \times 100$$

Where:

C: Mycelial growth of pathogen in control

T: Mycelial growth of pathogen in dual plate

Molecular Identification

The most potent inhibitory activity was chosen and identified for molecular identification of endophytic bacteria based on the sequencing of their 16S rRNA genes. The 16S rRNA gene was amplified using the universal primers 27F (5′-CAGAGTTTGATCCTGGCT-3´) and 1492R (5′-AGGAGGTGATCCAGCCGCA-3´) (**Li *et al.*, 2018**). The homology of a specific isolate's 16S rRNA sequence was determined using the BLAST-N programme from the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Scanning Electron Microscopy

Because several phytopathogens (including *R. solani*, *Alternaria* sp., and *F. oxysporum*) have undergone noticeable morphological changes, we need to be able to recognize them. Hyphae were preserved by fixing them, and then gold was coated onto them using an auto fine coater (JFC-1600) (**Yuan *et al.*, 1995**). The sample was then examined with a scanning electron microscope (**Jeol- jsm 5200**).

Antibiotic Susceptibility Assay

Antibiotic susceptibility of endophytic bacterial strains was determined using the Kirby-Bauer disc diffusion method (**Bauer and Wortzel, 1966**). After an overnight incubation in nutritional broth, sterile cotton swabs were used to inoculate Muller Hinton agar plates with all of the endophytic bacterial isolates. Standard antibiotic disks, such as ampicillin (10 µg/disk), vancomycin (30 µg/ disk), tetracycline (30 µg/disk), gentamycin (10 µg/disk), and Sulphmethoxazole (25 µg/disk), were placed on Muller Hinton agar plates and incubated 28 ^{0}C for 24 h. The zone of inhibition was determined by performing the experiment three times for each treatment.

In Vitro Screening for Plant Beneficial Traits

Indole acetic acid (IAA) production

Using Salkowski's colorimetric method, the ability of bacterial endophytes to produce indole -3-acetic acid (IAA) was determined. For 2–4 days at 28ºC with 125 rpm, Isolates were cultured in Nutrient broth supplemented with L-tryptophan (0.061 g l^{-1}) (**Bric *et al.*, 1991**). After incubation, the broth was centrifuged at 10,000 rpm for 10 minutes, and then 1 ml of the supernatant was combined with Salkowski reagent (0.5 M $FeCl_3$ dissolved in 35% perchloric acid) and incubated in the dark for 30 minutes. The appearance of pinkness indicated the formation of indole. After that, the OD at 530 nm was measured to check the findings. An IAA standard curve was utilized to derive the IAA concentration.

Phosphate Solubilization

On solid Pikovskya's medium supplemented with 5 g/L $Ca_3(PO_4)_2$, solubilization of inorganic phosphate was evaluated qualitatively for all endophytic bacterial strains (**Pikovskaya, 1948**).

The formation of clearing zones was assessed after seven days of incubation at 28°C. The presence of tricalcium phosphate in the agar medium was indicated by the formation of a colourless halo around the colonies. Quantitative estimation of phosphate solubilization was calculated according to the method described by **Jackson (2005)**.

Production of Siderophores

Iron (Fe) competition between ferric complexes of universal chrome azurol S (CAS) agar media was used to screen for siderophore synthesis, as described (**Alexander and Zuberer, 1991**). The incubation period for the isolates was 5-7 days at 28ºC. The formation of an orange halo zone around the colony and a noticeable shift in the medium's blue colour were taken as indicators of successful siderophore production.

Assays for proteolytic, cellulolytic, and chitinolytic activity

Spot inoculation on skim milk agar 5% (v/v) medium was used to test the bacterial strains for proteolytic activity. Incubation of the skim milk agar plates lasted for 48 hours at 28 ± 2ºC. The hydrolysis of skim milk was used to detect proteolytic activity (**Ntabo *et al.*, 2018**), as it produced a clear halo surrounding the bacterial colonies.

Cellulolytic activity was assayed with M9 medium (**Miller, 1972)**, which had been supplemented with yeast extract (1.2 g l^{-1}) and cellulose (10 g l^{-1}) by using the spot inoculation technique. Positive cellulase producing isolates were identified as those surrounded by clear halos after 8 days of incubation at 28 ± ºC (**Gao *et al.*, 2008**).

Chitinase activity was determined by plating samples onto chitin-agar plates and observing the results. Chitinase detection medium consisted of (L^{-1}) 0.5g NaCl, 6g M9 salts, 2g Chitin, 14.7g $Cacl_2$, 24.6g Mgso4, 1g Thiamin-HCl, 20g Agar. According to **Berg *et al.* (2000)**, chitinase activity was determined by the presence of a distinct halo during incubation at 28 ± 2 ^{0}C for eight days.

Evaluation of bioagents for the suppression of tomato root rot under greenhouse conditions

Soil was placed in plastic pots 20 cm in diameter and 35 cm in depth, and 200 ml of selected bacterial culture (*Lysinib. fusiformis* NAW3 and *Pseudomonas aeruginosa* NAW6) (10^8 CFU ml^{-1}) was thoroughly mixed with 2.8 kg of sterilized sandy soil for 2 minutes. Tomato seedlings were aseptically planted in plastic pots. Soil infestation was carried out using *R. solani* and *F. oxysporum* inoculum cultured on sterilised wheat kernels medium. Three days after sowing, 40 wheat kernels infected with the tested fungus were sown at a depth of approximately 2 cm, 3 cm apart from the tomato seedlings in each box. Each treatment was tested in six pots. The treatments were: (i) control, without pathogenic fungi and bacteria; (ii) plant with bacteria only; (iii) plant with pathogenic fungi only, and (iv) plant with pathogen and bacteria.

Evaluation

Disease severity

The severity of the disease was determined through observation by using disease classes described by **Pal *et al.*, 2001)**.

Effect of endophytic bacterial strains on plant growth

Three plants were measured per replication, giving each treatment a total of 9 plants. The root systems were carefully rinsed with water to remove clinging soil particles in order to determine plant fresh weight (g), plant height (cm), and plant dry weight (g). The plant was oven at 70 degrees Celsius for three days, and its dry weight (g) was measured.

Statistical Analysis

According to **Steel and Torrie (1981)**, preliminary data were statistically examined using the proper analysis of variance. The date of the experiment was randomly assigned with four replicates. Experiment data were analysed using the computer application CoStat, version 6.311. Least significant difference (LSD) at a 5% significance level was utilised to assess the reaction of each character in each experiment.

RESULTS

Isolation and Screening of Antagonistic Endophytic Bacteria

From the roots of diverse medicinal plants (rosmaria, maidenhair fern, saltbush, gum succory, aloe, and Egyptian henbane), bacterial endophytes

were isolated. On the basis of distinct colony morphologies on media, 300 isolates were obtained. Here, the only bacterial isolates capable of inducing a significant inhibitory zone in phytopathogenic fungi were considered antagonistic. Broad-spectrum antifungal activity was found in 18 of 300 bacterial isolates tested against phytopathogenic fungi (Fig. 1). The NAW6 isolate demonstrated the greatest antagonistic activity. The inhibition zone of mycelia growth for the examined fungi varied between 7.57 and 42.67 mm. The inhibition zone of fungal growth for *R. solani*, *F. oxysporum*, and *Alternaria* sp. were from 7.57 to 40.0 mm, 9.2 to 42.67 mm, and 10.4 to 42.5 mm, respectively (Fig. 2). Furthermore, the proportion of suppression of growth of the examined fungus ranged from 40% to 90.52%. Isolates NAW6 (90.52%), NAW9 (87.13%), NAW2 (86.97%), and NAW3 (86.67%) against *Rhizoctonia solani* than any other isolates. The most antagonistic isolates against *Fusarium oxysporum* were NAW6 (88.57%), and NAW8 (87.28%). When compared to other isolates, the isolates NAW3 (82.71%) was significantly the most efficient against *Alternaria* sp. (Fig. 3).

Identification of Bacterial Isolates

Eight endophytic bacterial isolates with strong antagonistic potential have been identified by analysing their 16S rRNA gene sequences. All of the isolates' 16S rRNA gene sequences matched those in the NCBI database with a degree of similarity ranging from 99.72% to 100% (Table 1). Following sequencing analysis, these bacteria were classified into six different families: *Enterobacteriaceae*, *Pseudomonadaceae*, *Caulobacteraceae*, *Morganellaceae*, *Paenibacillaceae*, and *Bacillaceae*. These bacterial isolates were classified into seven different genera (Table 1) based on the 16S rRNA gene sequencing data.

The isolates NAW4, and NAW7 had the closest genetic relationship with *B. pumilus* and *B. siamensis*, respectively. The isolate NAW2 and NAW10 showed similarity with *Paenib. peoriae* and *Paenib. polymyxa*, respectively. The isolate NAW3 had 99.39% similarity with *Lysinib. fusiformis*. The isolates NAW6 had the closest genetic relationship with *P. aeruginos*a. The isolate NAW8 had 100% similarity with *Providencia vermicola*. The NAW9 showed 99.72% similarity with *Brevundimonas diminuta* (Table 1).

Scanning Electron Microscopic Studies

Scanning electron microscopy has revealed potential microbial antagonistic interactions with the fungal pathogen. The presence of hostile bacteria resulted in a wide variety of morphological alterations in the mycelium, as contrasted with the control. Fig. 4 shows that there were several structural anomalies in the mycelium. Cell wall damage and nodal edoema were among the anomalies caused by *F. oxysporum*, *R. solani*, and *Alternaria* sp. Mycelia abnormalities may be caused by the formation of lytic enzymes and non-enzymatic antifungal compounds.

Evaluation of Bacterial Strains' PGP and Biocontrol Characteristics

The Selected strains were evaluated in vitro for multiple beneficial traits. The majority of isolates was able to produce ammonia as indicated by the yellow to brown color development (37.8%). Numerous isolates solubilized phosphate (50%), and synthesized IAA (62.5%). In accordance with quantitative analysis, the results revealed that NAW9 produced a high level of indole-3-acetic acid (IAA) (23.57 mg l^{-1}), followed by the strain NAW8 (19.49 mg l^{-1}). Strains NAW4, NAW6, NAW7, and NAW9 were the best P-solubilizers (Table 2). Many strains have the ability to produce siderophores (62.5%). Strains have more beneficial traits belonged to the genera *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Lysinibacillus*, and *Brevundimonas*. In addition, all the isolates were screened for the presence of digestive enzymes that may be involved in lysis of fungal pathogens. Table 2 displays that the majority of endophytic isolates produced at least one hydrolytic enzyme: cellulase (50%), protease (100%), and chitinase (37.5%). The endophytes producing at least two digestive enzymes belonged to the genera *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Brevundimonas*.

Antibiotic Susceptibility Assay

Endophytic bacterial strains were isolated from different medicinal plants and tested for antibiotic susceptibility using the disc diffusion method. Some of the endophytic bacteria were sensitive to the antibiotics that were tested (Table 3). The zone of inhibition around bacterial cultures showed varying degrees of

Fig. 1. Antagonism of endophytic bacterial isolates against fungal phytopathogens: (1) *Rhizoctonia solani*; (2) *Fusarium oxysporum*; (3) *Alternaria* sp. Growth inhibition of fungal mycelia was examined in dual culture assay on agar plate

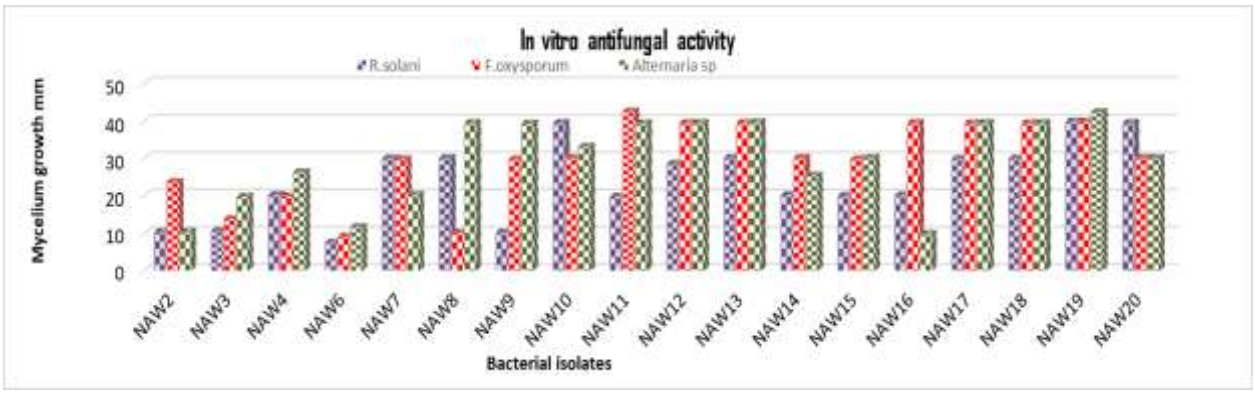

Fig. 2. Antagonistic effects of tested isolates against different phytopathogenic fungi as measured by inhibition of growth

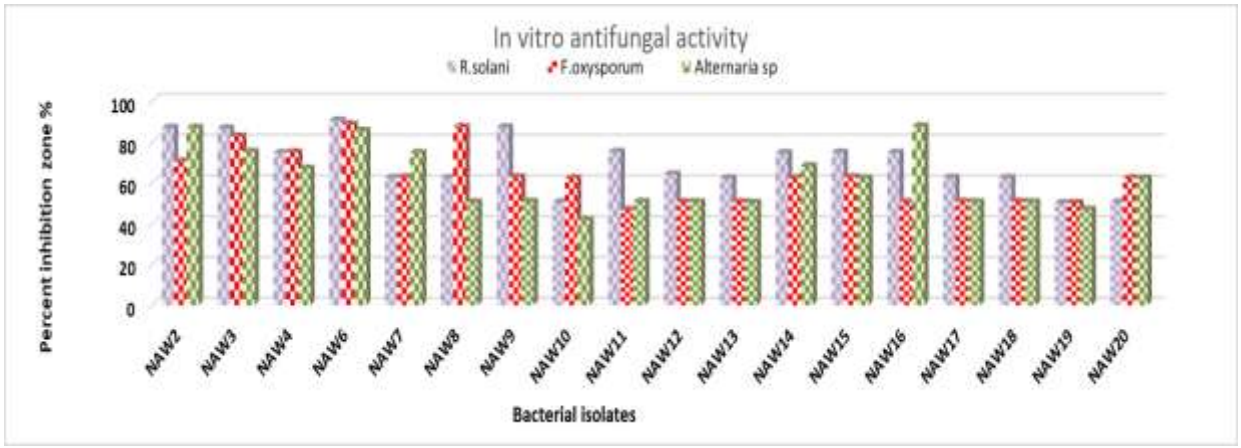

Fig. 3. Antagonistic effects of tested isolates against different phytopathogenic fungi as measured by inhibition of mycelium growth

Table 1. Identification of endophytic bacterial isolates by 16S rRNA gene sequencing

Isolate code	NCBI closest match With Accession no.	Similarity
NAW2	*Paenibacillus peoriae* MN198021.1	99.88%
NAW3	*Lysinibacillus fusiformis* MT605500.1	99.39%
NAW4	*Bacillus pumilus* MK501617.1	99.84%
NAW6	*Pseudomonas aeruginosa* MF599303.1	99.84%
NAW7	*Bacillus siamensis* MK373318.1	99.82%
NAW8	*Providencia vermicola* MN601273.1	100%
NAW9	*Brevundimonas diminuta* MT527531.1	99.72%
NAW10	*Paenibacillus Polymyxa* CP097769.1	100%

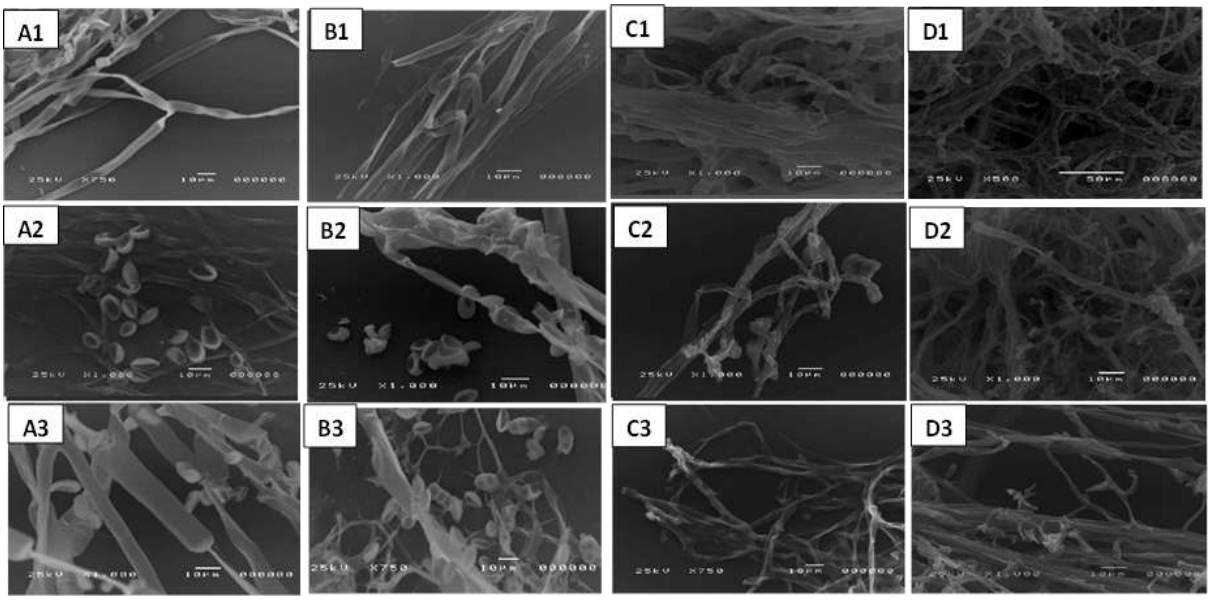

Fig. 4. Scanning electron microscopic photographs showing cell wall damage and bacterial colonization by antagonistic effects of isolates on, *Fusarium oxysporum*, and *Alternaria* sp; (A1 and B1) *R. solani* without antagonistic (control), (A2 and B2) *F. oxysporum* without antagonistic (control), (A3 and B3) *Alternaria* sp without antagonistic (control), (C1 and D1) *R. solani* with antagonistic,), (C2 and D2) *F. oxysporum* with antagonistic, and (C3 and D3) *Alternaria* sp. without antagonistic

Table 2. Plant growth promoting activities and biocontrol characteristics of antagonistic endophytic bacteria

Isolates	P solubilization (mg P l^{-1})	IAA production (mgl^{-1})	Siderophores production	Ammonia production	Cellulase production	Protease production	Chitinase production
NAW2	-	-	-	-	-	++	-
NAW3	-	14.36	++	-	-	+++	-
NAW4	153	18.06	+	++	+++	++	-
NAW6	123	17.98	++	+++	+	+++	-
NAW7	113	-	+	-	-	+	++
NAW8	-	19.49	-	-	+++	+	+
NAW9	108	23.57	+++	++	-	++	++
NAW10	-	-	-	-	+++	+	-

Table 3. Dimeter of the growth inhibition zone of endophytic bacteria against multiple antibiotics

Bacterial srtains	Sulphmethoxazole 25	Gentamycin 10	Vancomycin 30	Tetracycline 30	Ampicillin 10
	Zone of inhibition (mm)				
***Paenibacillus peoriae* NAW2**	27.3^{c}	17.4^{d}	20.7^{e}	22.9^{c}	$-ve^{c}$
***Lysinibacillus fusiformis* NAW3**	6.1^{f}	12.4^{e}	$-ve^{e}$	$-ve^{e}$	$-ve^{c}$
***Bacillus pumilus* NAW4**	$-ve^{h}$	10.5^{f}	19.3^{d}	11.4^{d}	$-ve^{c}$
***Pseudomonas aeruginosa* NAW6**	34.5^{a}	22.3^{a}	24.7^{b}	26.6^{b}	10.4^{a}
Bacillus siamensis NAW7	$-ve^{h}$	19.4^{c}	$-ve^{e}$	$-ve^{e}$	$-ve^{c}$
***Providencia vermicola* NAW8**	20.2^{e}	11.3^{d}	$-ve^{e}$	$-ve^{g}$	$-ve^{c}$
***Brevundimonas diminuta* NAW9**	33.6^{b}	20.6^{b}	29.5^{a}	26.6^{b}	$-ve^{c}$
***Paenibacillus Polymyxa* NAW10**	25.2^{d}	19.6^{c}	21.1^{c}	33.4^{a}	6.3^{b}
	0.226	0.387	0.263	0.234	0.093

resistance and sensitivity to each antibiotic. There was no Gentamycin resistance seen among any of the bacterial strains. With the exception of *Providencia vermicola* and *B. siamensis*, all tested bacteria were susceptible to Sulphmethoxazole. Tetracycline resistance has been demonstrated in *Paenib. polymyxa*, *B. siamensis*, and *Brevundimonas diminuta*. *B. pumilus*, and *Lysinib. fusiformis* exhibited no resistance to Ampicillin in the tests. All of the bacterial strains were sensitive to Vancomycin with the exception of *Paenib. polymyxa*, *B. siamensis*, and *Brevundimonas diminuta*.

Plant Experiments

To determine whether endophytes promote plant growth and biocontrol, two bacterial strains, *B. pumilus* NAW4 and *P. aeruginosa* NAW6, positive for at least three plant-beneficial traits, were chosen to test their plant growth stimulation properties in pot experiments with tomato plants in the presence of a phytopathogenic fungus. It is clear from Table 4 that infected tomato plants with *R. solani* and *F. oxysporum* showed significant reductions in plant length (34-.30%), fresh plant weight (42.38-38.14%), and dried plant weight (64-58%), respectively compared to healthy control

Table 4. Effect of endophytic bacterial strains on plant growth of tomato plants with two different phytopathogenic fungi under greenhouse experiment

	Plant length (cm)		fresh weight (g)		dry weight (g)		Disease severity	
treatments	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
T1	49.73^{e}	0.05	24.83^{d}	0.05	5.65^{e}	0.01	0.64d^{d}	0.01
T2	45.29^{g}	0.10	22.24^{e}	0.05	4.27^{f}	0.01	0.83^{c}	0.01
T3	57.59^{b}	0.25	33.27^{b}	0.06	6.12^{d}	0.01	0.00^{fg}	0.01
T4	52.43^{c}	0.09	26.87^{c}	0.06	6.88^{b}	0.07	0.52^{f}	0.00
T5	48.55^{f}	0.05	24.55^{d}	0.12	5.50^{f}	0.10	0.56^{e}	0.00
T6	61.94^{a}	0.05	33.75^{a}	0.05	7.87^{a}	0.05	0.00^{g}	0.00
T7	33.46^{h}	0.05	14.53^{f}	0.07	2.24^{i}	0.10	2.44^{b}	0.10
T8	35.35^{i}	0.05	15.60^{g}	0.10	2.63^{g}	0.06	3.23^{a}	0.10
T9	50.42^{d}	0.49	25.22^{cd}	0.10	6.24^{c}	0.04	0.00^{g}	0.00
L.S.D $_{0.05}$	0.295		2.080		0.115		0.239	

Values followed by the same letters at the same column are not significantly different by LSD's test at 0.05 level.

- (**T1**) infected plants with *R. solani* treated with *Pseudomonas aeruginosa* NAW6
- (**T2**) infected plants with *F. oxysporum* treated with *Pseudomonas aeruginosa* NAW6
- (**T3**) healthy plants treated with *Pseudomonas aeruginosa* NAW6
- (**T4**) infected plants with *R. solani* treated with *Bacillus pumilus NAW4*
- (**T5**) infected plants with *F. oxysporum* treated with *Bacillus pumilus* NAW4
- (**T6**) healthy plants treated with *Bacillus pumilus* NAW4
- (**T7**) plants infected with *Rhizoctonia solani* (infected control 2)
- (**T8**) *F. oxysporum*-infected plants (infected control 1)
- (**T9**) untreated plants (healthy control)

plants. The results revealed that various growth parameters (shoot length, root length and dry plant weight) were significantly increased by using endophytic bacterial strains, as shown in Table 4. On the other hand, infected plants treated with selected endophytic bacterial strains showed hopeful recovery, and the best treatment was *P. aeruginosa* NAW6.

DISCUSSION

In this study, 300 isolates were isolated from the roots of different medicinal plants that grew in the Sinai desert, where conditions were harsh. All of the medicinal plants that were examined for this study were found to be colonised at least by one entophytic bacterial species. These are expected results, Microbial symbionts are found in almost all plants under normal or stressful conditions, and many plant species have hundreds of endophytes (**Singh *et al.*, 2011; Szymańska *et al.*, 2016)**. Numerous previous studies have investigated the variety of bacterial endophytes in medicinal plants (**Adamović *et al.*, 2015; Castronovo *et al.*, 2020; Abdel-Sater *et al.*, 2021; Hamayun *et al.*, 2021; Yadav and Meena, 2021; Mousavi and Karami, 2022**).

The only bacterial isolates capable of inducing a substantial inhibitory zone in phytopathogenic fungi were chosen for this investigation. In the end, only eight of the selected and examined isolates demonstrated significant broad-spectrum

antifungal activity. Eight bacterial endophytes were identified as different species of *Bacillus*, *Paenibacillus, Lysinibacillus*, *Proteus*, *Providencia*, and *Brevundimonas*. According to **Zam *et al.* 2019)**, a variety of endophytic bacteria from traditional medicinal plants possess anti-phytopathogenic properties. Our results are similar to those of **Yasmin *et al.* (2014), Sasirekha and Srividya (2016) and Huang *et al.* (2012)**, who reported *Pseudomonas* isolate have significant antagonistic effects against numerous organisms, including *Fusarium oxysporum*, *Rhizoctonia solani*, *F. graminearum*, *Phytophthora capsici*, and *Pythium ultimum*. From the native desert medicinal plant *Teucrium polium* L., **(Hassan, 2017)** isolated six bacterial endophytes, including *B. cereus* and *B. subtilis*, with varying degrees of broad-spectrum activity.

As a result, we evaluated the endophyte collection *in vitro* for beneficial plant features with the goal of identifying the most promising microbes. Similar studies found that endophytic bacteria demonstrated a wide variety of plant-beneficial properties, including the synthesis of plant growth hormones, nitrogen fixation, and phosphate solubilization (**Liu *et al.*, 2016; Egamberdieva *et al.*, 2017; Paul and Sinha, 2017; Li *et al.*, 2018b; Afzal *et al.*, 2019**). Several endophytic bacteria showed the potential of plant growth promotion through the production of one more activities, such as ammonia production **(Li *et al.*, 2018a; Sajjad *et al.*, 2021**), IAA production (**Alkahtani *et al.*, 2020; Laird *et al.*, 202**0), phosphate solubilization (**Mihoub *et al.*, 2021; Yahya *et al.*, 2021; Adnan *et al.*, 2022; Khan *et al.*, 2022**), or siderophores production (**Boiteau *et al.*, 2016; McRose *et al.*, 2018; Garg *et al.*, 2021**).

The bacterial endophytes exhibited different enzymatic activities involving cellulase, pectinase, protease, amylase, and chitenase production **(Eid *et al.*, 2019; Fouda *et al.*, 2019**). Specifically, cell wall degrading enzymes are very important for controlling the phytopathogenic fungi such as chitinase, cellulase and proteases. Depending on their enzymatic activity, bacterial endophytes can give the plant with nutrients and defend it from phytopathogen infection by secreting a variety of lytic enzymes **(Passari *et al.*, 2016**). Cellulolytic activities are known to enable microorganisms to penetrate plant tissues and establish a symbiotic relationship with their host plants. Previous results showed that many bacterial endophytes exhibited cellulase production (**Eid *et al.*, 2019; Fouda *et al.*, 2019; Alkahtani *et al.*, 2020**). chitinolytic are responsible for the destruction of cell walls, and chitinases secreted by PGPR inhibit the growth of harmful fungal hyphae by degrading the primary component of their cell wall (**Goswami *et al.*, 2016; Munir *et al.*, 2018; Malik, 2019**). Previous results demonstrated that protease enzymes released by bacterial endophytes are crucial for protecting plants from damage caused by pathogens **(Hassan, 2017; Morales-Cedeño *et al.*, 2021**). There are various previous results showed that many bacteria could produce protease (**Ali *et al.*, 2020c; Bhattacharyya *et al.*, 2020a; Jadhav *et al.*, 2020; Vandana *et al.*, 2021**).

After understanding the antagonistic interactions in vitro, the next step is to validate these interactions under green conditions. The microbial combinations for the present study's evaluation were chosen based on the outcomes of the in vitro experiments. Tomato (*S. lycopersicum* L.) is one of the world's most important and extensively cultivated plants. For the management of plant diseases, however, the most effective strains were subjected to in vivo bioassays that simulated actual plant–soil–microbe interactions under controlled conditions to determine the effect on plant growth and the control of fungal pathogens. The significance of NAW3 and NAW6 in promoting plant growth can be attributed to PGPR's direct effect on increasing nutrient availability, phytohormone production, and water assimilation (**Elhelaly, 2022; Rizvi *et al.*, 2022**). In addition, indirect action of PGPB can promote plant growth by preventing or inhibiting the growth or activity of phytopathogens through a variety of mechanisms, such as competition for space and nutrients, antibiosis, lytic enzyme production, toxin inhibition, and induction of plant defence mechanisms (**Solanki *et al.*, 2019; Tapia-Vázquez *et al.*, 2020**). These research findings are in agreement with the current study because of the tested two strains (*Lysinib. fusiformis* NAW3 *and P. aeruginosa* NAW6) have ability to produce IAA, ammonia, siderophores, chitinase, protease, and solubilize insoluble inorganic phosphate. The use of PGPR in disease suppression and plant growth

promotion (PGP) is a widely adopted strategy in various crops such as wheat **(Abbasi *et al.*, 2011**), pepper (**Mannai *et al.*, 2020**), rice (**Yasmin *et al.*, 2016**), okra (**Begum *et al.*, 2012; Bhattarai *et al.*, 2022**), cucumber (**Islam *et al.*, 2016**), potato **(Kenawy *et al.*, 2019**), and tomato (**Szczech and Shoda, 2004; Alsudani, 2020; Gaya Karunasinghe *et al.*, 2020; Abreo *et al.*, 2021**). Rhizobacteria belonging to *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Arthobacter*, *Bacillus*, and *Serratia* spp. enhance plant growth, and are being used as bio-controls (**Labuschagne *et al.*, 2010; El-Sayed *et al.*, 2014; Ganapathy and Natesan, 2018; Attia *et al.*, 2020**).

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استكشاف إمكانيات البكتيريا الداخلية المضادة المعزولة من النباتات الطبية وتأثيرها المثبط على أمراض الفطريات التي تنتقل عن طريق التربة في الطماطم

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تستضيف النباتات الطبية مجموعة متنوعة من الميكروبات الداخلية التي تحمل قيمة اقتصادية كبيرة. يركز هذا البحث على عزل وتحديد البكتيريا الداخلية من النباتات الطبية التي تنمو في المنطقة الجافة من العريش (مصر) ودورها المحتمل كعوامل تعزيز حيوية لتحسين نمو نباتات الطماطم. في هذه الدراسة، أظهرت ثمانية عزلات بكتيرية داخلية نشاطًا واسع المجال في المعمل ضد الفطريات المختبرة. بناءً على النشاط المضاد للفطريات، تمت دراسة هذه العزلات وتحديدها بناءً على تسلسل جين 16S rRNA، حيث تم تحديدها كـ *Lysinibacillus fusiformis*، *Bacillus pumilus*، *Bacillus siamensis*، *Paenibacillus peoriae*، *Paenibacillus polymyxa*، *Pseudomonas aeruginosa*، *Brevundimonas diminuta*، و *Providencia vermicola*. تم فحص السلالات من حيث خصائص تعزيز نمو النباتات (PGP) المتعددة، بما في ذلك إنتاج حمض الأندول-3- خليك (IAA) ، الأمونيا، السايدروفورات، الفوسفاتاز، إنتاج الإنزيمات التحليلية، وذوبان الفوسفات. أظهرت السلالات البكتيرية المعزولة قدرات متنوعة في تعزيز نمو النباتات. تم تقييم سلالتين مختارتين من البكتيريا الداخلية لدراسة قدرتها على المكافحة الحيوية ضد مرض تعفن الجذور الفطري في الطماطم الذي يسببه *Fusarium oxysporum* و *Rhizoctonia solani* وذلك لتقييم قدراتها على تعزيز النمو تحت ظروف الصوبة. تحت ظروف الصوبة، أثبتت *Bacillus pumilus NAW4* و *Pseudomonas aeruginosa NAW6* فعالية في تقديم فوائد إيجابية للطماطم تحت ظروف الإجهاد وكذلك في ظل الظروف الطبيعية للنمو.

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