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Diversity, Characterization, and Potential Applications of Bacterial Endophytes Isolated from the Halophyte *Limonium axillare*

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Abstract
Recently, there has been a worldwide call to explore nature-friendly compounds, which could enhance plant growth and substitute for chemically synthesized products. Endophytes are a group of microorganisms that lives in the plants and algae symbiotically. In this research, endophytes were isolated from leaves of the halophyte, *Limonium axillare*. A total of 280 bacterial isolates were obtained from the leaves of *L. axillare*. Strains displaying similarities in terms of morphology and biochemical reactions were categorized into 48 groups. One representative from each group was identified and subjected to enzymatic and plant growth-promoting tests. Forty-eight isolates were identified using a sequence similarity-based method based on the 16S rDNA gene. The identified strains were categorized into two genera: *Bacillus* and *Staphylococcus*. Our investigation uncovered 44 isolates from the *Bacillus* genus, representing 10 different species, including *Bacillus* sp., *B. pseudomycoides*, *B. cereus*, *B. paramycoides*, *B. velezensis*, *B. subtilis*, *B. thuringiensis*, *B. wiedmannii*, *B. anthracis*, and *B. bacterium*. Furthermore, we observed that only 4 isolates belonged to the *Staphylococcus* genus, encompassing 3 distinct species: *S. bacterium*, *S. succinus*, and *S. saprophyticus*. The isolated bacteria were screened for extracellular enzymes, plant growth promoting traits, antifungal activity, and salinity tolerance. From the previous screening, diverse responses were obtained. Most of the isolates were secreted at least one of the hydrolysis enzymes (protease, lipase and amylase). (93.8%) of the strains showed phosphate solubilization activity. (33.3%) produce (IAA). Siderophore production potential was present in 91.7% of isolates, while ACC deaminase and HCN production activities were identified in 52.1 and 41.7% of strains, respectively. Additionally, DNase activity was evident in 27.1%, and ammonia production was observed in 31.3% of the isolates. The isolates *Bacillus velezensis* (AL4QUA) strain showed positive effect in the greenhouse experiment in terms of plant growth promoter agent and biocontrol agent against *Fusarium oxysporum* pathogen of tomato seedling. Thus, endophytes have the potential to reduce chemical inputs in conventional agricultural practices, increase nutrient uptake and improve plant stress resilience.

Keywords Endophytes · Halophytes · PGPB · Biocontrol · Biofertilizer

Introduction

Halophytes are plants uniquely adapted to thrive in environments with elevated salt concentrations, showing a remarkable ability to tolerate high levels of salt. They have adapted to thrive in these conditions by developing specialized survival strategies (Flowers and Colmer 2008). One intriguing aspect of their adaptation is their association with endophytic microorganisms (Rodríguez-Llorente et al. 2019). Endophytes are microorganisms, including bacteria and fungi that reside within the internal tissues of plants without causing harm (Hallmann et al. 1997). These endophytes play a crucial role in enhancing the growth, stress tolerance, and overall fitness of halophytes to saline environments (Komaresofla et al. 2019). The study of halophyte-endophyte interactions, as well as its potential application in the field of sustainable agriculture, has received significant attention in recent years.

Endophytes play a key role in halophytes, but research has recently shed light on the diversity and functions of these

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organisms. The production of osmoprotectants, the promotion of nutrient uptake, or the modulation of plant hormone levels have been documented as mechanisms by which endophytic bacteria contribute to salt tolerance (Saberi Riseh et al. 2021; Mahgoub et al. 2021; Afridi et al. 2019). Additionally, fungal endophytes have been shown to enhance the production of secondary metabolites in halophytes, including antioxidants and antimicrobial compounds (Calado et al. 2021; Andhale 2021), which could have valuable applications in pharmaceuticals and biotechnological industries.

The interaction between halophytes and endophytes is not limited to salt tolerance alone. Endophytes have also been shown to confer resistance against various biotic stresses, including pathogen attacks and herbivory, thereby improving plant health and survival (Christakis et al. 2021; Singh et al. 2021; Ajijah et al. 2023). Furthermore, the presence of endophytes in halophytes has been linked to improved nutrient acquisition, water use efficiency, and overall plant growth (Ajijah et al. 2023; Choudhury et al. 2021).

A plant growth promotion (PGP) strategy involves the interaction of plants with certain microorganisms, such as bacteria, fungi, and archaea, that contribute to the health or development of plants (Compant et al. 2010). The PGP microorganisms establish symbiotic or associative relationships with plants and provide them with a range of benefits, such as nutrient acquisition, hormone production, disease suppression, and stress tolerance (Glick 2012). Over the past decade, PGP microorganisms have gained considerable attention as a promising approach to improving agricultural productivity and sustainability (Mishra et al. 2017; Omomowo and Babalola 2019). Among the primary mechanisms by which PGP microorganisms promote plant growth is by increasing nutrient availability and uptake (Glick 2012). Some rhizobacteria, such as *Azospirillum* and *Pseudomonas*, have been shown to solubilize phosphorus and increase its availability for plants (Robin et al. 2008). Leguminous plants benefit from symbiotic relationships with nitrogen-fixing bacteria such as *Rhizobium* and *Bradyrhizobium* (Giraud et al. 2007). In addition, PGP microorganisms produce hormones, particularly phytohormones that contribute to plant growth and development (Kurepin et al. 2014). The production of indole-3-acetic acid (IAA) by certain bacteria promotes root development and nutrient uptake (Chhun et al. 2004). Other microorganisms, such as mycorrhizal fungi, produce gibberellins and cytokinins, which stimulate plant growth and flowering (Bona et al. 2015).

Plants possess diverse strategies to be protected from pathogens, including antagonistic interactions and the activation of mechanisms such as induced systemic resistance (Bae et al. 2011). Among the microorganisms capable of protection, plants against pathogens are endophytes (Afzal et al. 2019). Due to their location within the plant cell, these microorganisms are more protected from environmental stresses than Rhizobacteria are (Rana et al. 2020; Kuklinsky-Sobral et al. 2004). This unique characteristic has prompted extensive research into their potential as biocontrol agents. Notably, recent findings have also highlighted their ability to enhance plant growth and overall health (Ji et al. 2014).

The endophytes hold promise as a potential biological control agent for bacterial and fungal pathogens, specific genera such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Burkholderia*, and *Agrobacterium* have received predominant attention in both research and commercial applications (Fravel 2005; Arguelles-Arias et al. 2009; Cawoy et al. 2015; Cao et al. 2018). In addition to proteins, peptides, lipopeptides, bacteriocins, and secondary metabolites, these microorganisms produce various compounds that have antibacterial activity against phytopathogens (Montesinos 2007; Zhao et al. 2014). Purification and characterization of each of these bioactive compounds can be accomplished by specific procedures.

Research has indicated that endophytic bacteria (EB) associated with halophytes can serve as valuable and environmentally friendly supplements to enhance the growth of plants, both halophytic and non-halophytic, in saline soils (Etesami and Beattie 2018). Consequently, there is a growing interest in exploring and harnessing the potential of endophytic resources linked to halophytes (Sofy et al. 2021; Kearl et al. 2019).

Fusarium oxysporum, a soil-borne pathogen, is responsible for causing extensive damage in multiple crop types. It is noteworthy that *F. oxysporum* is listed among the top ten economically harmful fungi, leading to substantial agricultural losses in crops such as banana, cotton, canola, and tomato, as documented by Dean et al. (2012). Employing broad-spectrum fungicides to control this fungus has led to environmental pollution and the development of resistance. As a more environmentally friendly alternative, the exploration of safe bio-control agents is highly recommended in the management of phytopathogene infections (Zheng et al. 2016).

Limonium axillare, (Forssk.) Kuntze, commonly known as the Mediterranean Sea lavender, is a halophytic plant species that belongs to the family Plumbaginaceae. It is native to coastal regions of the Mediterranean and exhibits remarkable adaptations to high salinity and arid conditions (Fig. 1). In recent years, there has been increasing interest in studying *L. axillare* due to its ecological significance, unique physiological adaptations, and potential applications in various fields. To the best of our knowledge, this study is the first in its kind to isolate the bacterial endophytes from the halophyte *L. axillare*. Thus, the present study focuses on the isolation, purification, identification and biochemical characterization of any bacterial endophytes associated with the leaves of the halophyte *L. axillare*.

Fig. 1 *Limonium axillare* a halophytic plant (Location: Al Thakhira—Qatar)

Our current study focused on four primary objectives: The first objective was to isolate bacterial endophytes from halophytic plants, aiming to uncover unique microbial strains thriving in these specific ecological niches. is the second objective was to conduct biochemical tests to evaluate the plant growth-promoting (PGP) potential of the isolated bacterial endophytes by subjecting the isolates to various assays, we aim to identify and characterize strains that exhibit significant capabilities in promoting plant growth. The third objective was to investigate the antagonistic properties of the isolated bacterial endophytes against fungal pathogens. Understanding how these microbes interact with and potentially control fungal pathogens is essential for exploring their role in biocontrol strategies. The fourth objective was to assess the specific impact of selected isolates on *Fusarium oxysporum*, a notable fungal pathogen. Moreover, preliminary observations indicate the growth-promoting effects of a highly efficient isolate on tomato plants exposed to the fungal pathogen *F. oxysporum* infection.

Materials and Methods

Study Sites and Sample Collection

The state of Qatar is located in the Arabian Gulf region (50° 45′–51° 40′ E longitude and 24° 40′–26° 10′ N latitude) with an area of approximately 11,400 square kilometers. This region is characterized by warm and hyper-arid desert ecosystem. Qatar is hot and has dry weather; therefore, the temperature could exceed 45 °C during the summer months from July to August. *Limonium axillare* (Forssk.) Kuntze is native to Qatar and not endangered or threaten species. In the present study, the plant *L. axillare* was collected from two locations Al Thakhira coastal area (25° 42′ 4″ N, 51° 33′ 16″ E) and Qatar University protected field (25° 22′ 6″ N; 51° 29′ 35″ E). *L. axillare* was collected after proper authorizations and all methods were carried out in following relevant regulations. To isolate endophytes, 35 healthy plants were selected for sampling. Ten leaves from the middle of eachplant sample were collected (Total 350 leaves), stored properly in labeled plastic bags in an icebox, transferred to the lab, and stored in a refrigerator at 4 °C. The isolation process conducted within 0–24 h after collection.

Isolation of Endophytes

The collected leaf samples were cleaned by tap water to remove dust and any debris present followed by washing for several times with autoclaved distilled water. After that the leaves were surface-sterilized using methods as follows: leaf samples were immersed in sodium hypochlorite (5%) for 2 min, then immersed in sodium ethanol (70%) for 5 min, followed by washing in sterile distilled water for several times to eliminate agents of the sterilization process. The samples were then cut into pieces under aseptic conditions and the leaf pieces were placed into Petri dishes containing Nutrient Agar media. The plates then incubated at 30 °C for 24–48 h. The emerging endophytes were transferred onto new Petri plates for the purpose of purification and storage. The purified strains were preserved in 30% (v/v) glycerol and kept at − 20 °C for further use and long-term storage. To confirm the efficiency of the sterilization process in killing all epiphytic bacteria, an aliquot of sterile distilled water from the final rinse was plated on nutrient agar plates and incubated under similar conditions. If there is no microbial growth, then the leaf samples were considered surface sterilized. The endophyte colonization rate was calculated according to the following equation (Petrini and Carroll 1981).

$$\text{Colonization rate } \% = (\text{number of segments colonized by an endophytes} / \text{total number of segments}) \times 100$$

Molecular Identification of Endophytes

The endophytic microbes were identified molecularly by genomic DNA (deoxyribonucleic acid) extraction, PCR techniques, nucleotide sequencing, and phylogenetic analysis.

Isolates were grown overnight on nutrient agar medium to obtain pure cultures. DNA was extracted from cells by thermal lysis (0.5 mL water suspension, boiling for 15 min, centrifugation for 4 min at 14 000 rpm). The supernatant from this reaction was used to amplify the PCR product. Amplification of the 16S rRNA fragment was done using primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′- GGTTACCTTGTTACGACTT-3′). Amplicons were purified after amplification and sequenced by a sanger sequencer (3130/3130xl DNA Analyzers). The BLAST algorithm was used to find identical or related sequences in the NCBI (National Center for Biotechnology Information) Gene Bank database.

In Vitro Analysis of Hydrolytic Enzymes

All the endophytic bacterial isolates were screened for 3 enzymes (protease, lipase, and amylase). Skim milk agar medium was used to assess protease activity (van den Berg et al. 1993). For screening of amylase activity, the isolates were inoculated on starch agar plates (Dunican and Seeley 1962). In the case of lipid hydrolysis, tributyrin agar were used to detect the activity of lipase (Smeltzer et al. 1992). The assessments of the above enzymes were performed in triplicate for each assay.

Assessment of Characteristics of Plant Growth Promotion

An assessment of the growth-promoting traits of bacterial isolates was conducted based on the production of ammonia, the solubilization of inorganic phosphate, the production of siderophore, the production of IAA, the nitrogen fixation and the activity of ACC deaminases. The assessment of each activity was performed in triplicate for all assays.

Analysis of ammonia production was performed using a qualitative method. Bacterial isolates were tested for their ability to produce ammonia in peptone water. Each tube was inoculated with 10 mL peptone water using fresh-grown cultures and incubated for 48–72 h at 28 °C. In each tube, Nessler's reagent (0.5 mL) was added. In a positive test for ammonia production, brown to yellow coloration was observed.

In order to determine the solubilization capacity of inorganic phosphate, bacteria isolates were spot-inoculated on modified Pikovskayas agar plates (mended with bromophenol blue dye) using tricalcium phosphate. After 7 days of incubation at 28 °C, phosphate solubilization was determined by the appearance of transparent halo zones around bacterial colonies.

For siderophores production assay. CAS blue agar plates were inoculated with 24 h old bacteria cultures to determine the production of siderophores. Incubation has been carried out for 7 days at 28 °C on CAS blue agar plates. The formation of orange zones around the growing colonies was indicative of the production of siderophores.

The screening of indole acetic acid (IAA) was carried out by inoculating one loop of 24 h old culture into 5 mL Tryptone water and incubating at 30 °C with shaking at 125 rpm for 2 days. After incubation, 1 mL of Kovac's Indole reagent was added to the tube and mixed gently. The formation of bright pink color in the top layer indicates the positive result for indole.

ACC deaminase activity was determined by inoculating fresh bacterial isolates on the minimal agar plates amended with 3 mM ACC instead of $(NH_4)_2SO_4$ as the sole nitrogen source. The inoculated plates were incubated at 30 °C for 3 days and growth was monitored daily. Colonies growing on the plates were considered ACC deaminase producers.

To investigate the potential of the bacterial isolate to be capable of nitrogen fixation, the fresh bacterial isolates were streaked on Norris glucose nitrogen free media and kept at 30 °C for 2 days to observe the growth. The colonies formed after incubation were transferred twice to the same medium plates in the same conditions to reconfirm their nitrogen fixation.

In Vitro Antagonism Assessment

Twenty-four bacterial isolates, which shows at least four positive results of plant growth promoting characteristics the antagonistic activity was evaluated using the dual culture method (Ganesan and Gnanamanickam 1987). Individual bacterial strains and fungi were co-cultured on Potato Dextrose Agar (PDA) medium. A mycelial plug with a diameter of 5 mm was obtained from the periphery of a 3-day-old fungal culture on PDA. This plug was positioned at the center of a 90 mm Petri dish. On two opposing sides of the plug, 24-h-old cultures of each bacterial strain were streaked in a straight line, maintaining a distance of 2.5 cm from the plug, ensuring that the bacterial streaks ran parallel to each other. Petri dishes without bacterial streaks were used as controls. The Petri dishes were then incubated at 25 °C. Each treatment was replicated 3 times. After 10 days of incubation, the fungal growth diameter was measured, and the percentage of mycelium inhibition over the control was calculated using the following formula.

$$\% \text{ Mycelium inhibition} = ((C - T)/C) \times 100$$

here, 'C' represents the fungal mycelium's maximum growth under control conditions, and 'T' represents the fungal mycelium growth in the treatment.

Tomato Plant Bioassay

Tomato Seeds Growth Conditions

Seeds of tomato *Solanum lycopersicum* L. (Alta, Pomodoro marglobe, Italy) were purchased from the local market. To promote seed germination and eliminate pathogens, the seeds were surface-sterilized by immersing them in sodium hypochlorite solution (5%) for 2 min and then ethanol (70%) for 3 min followed by rinsing with sterilized distilled water for about 3–5 min. Tomato seeds were planted in a tray filled with a 50% peat moss and 50% soil mixture that had been autoclaved. The tray received regular irrigation with tap water and was placed in a greenhouse environment (at 20 ± 2 °C with and a minimum photon flux density of 350 ± 50 µ moL m^{-2} s^{-1}). After approximately 2 weeks from the initial sowing, the germinated seedlings were individually moved to 10 cm diameter plastic pots. These pots contained a sterile blend of peat moss and soil in a 1:1 ratio.

Bacterial Endophytic and Pathogenic Fungi Inoculum Preparation

The antagonistic bacterial strain (AL4QUA) was cultured on NA plates for 48 h. Subsequently, two loopful of a fresh bacterial culture were added to 5 mL of sterile distilled water and incubated at 37 °C for 30 min before application. In the pot experiment, each pot requiring bacterial treatment received 10 mL of a bacterial suspension (AL4QUA) in sterile distilled water with an optical density (OD600) of approximately 0.4. This treatment was carried out 1 week prior to inoculating the phytopathogenic fungi. The *Fusarium oxysporum* strain, which was supplied by Qatar University's Department of Biological and Environmental Sciences. Cultivation of the *F. oxysporum* fungus was carried out on (PDA) medium for 1 week at 25 °C. The pathogen inoculum concentration, adjusted to roughly 10^8 spore/mL, was determined with the aid of a hemocytometer.

Greenhouse Experiment

2-week-old seedlings of tomato were transplanted in 10 cm diameter pots. Then, the plantlets were inoculated with bacterial endophyte (AL4QUA) and kept under greenhouse conditions. The experiment was one factor with four treatment levels in a completely randomized design and four replications. The treatments were as follows: (1) Untreated seedlings, (2) seedlings inoculated with bacterial endophyte (AL4QUA), (3) seedlings inoculated with the pathogen *Fusarium oxisporum* spore suspension and (4) seedlings that have been inoculated first (1 week earlier) with bacterial endophyte (AL4QUA) and then inoculated with the plant pathogen *F. oxisporum*. The last treatment mainly used to detect possible antagonism between the endopyte activity and the plant pathogen. No fertilizer or pesticide was used. The plant growth was observed daily up to 6 weeks for any disease symptoms. Weekly, leaf chlorophyll contents (using SPAD 502 Plus Chlorophyll Meter) and plant heights were measured. 6-weeks post treatment (harvesting time) all plants were separated from the soil and their roots were softly washed from soil deposits, and each plant were separated into two portions, the belowground (root) and the aboveground (shoot). Plant parts were placed separately in paper bags, oven dried at 80 °C for 72 h and then the dry weight (biomass) were measured.

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) for the data, and statistical significance was attributed to P-values of ≤ 0.05. To make mean comparisons, Tukey's test at a significance level of $P \leq 0.05$ was performed, either between control and treated groups or among all treatment categories. Furthermore, Principal Component Analysis (PCA) was employed in order to assess the correlation between the microorganisms and the parameters measured in the tests. All data analyses were performed using OriginPro (Version 2023; OriginLab Corporation, USA).

Results

Isolation of Bacterial Endophytes

In this study, bacterial endophytes were isolated from fresh leaves of the halophytic plant *Limonium axillare*. Sterilization of the surface of sample explants was crucial for removing epiphytic microbes. Due to the absence of growth on the control plate, this step was satisfactory in our study. An adequate number of endophytes were observed on the edges of explants (leaf) on LB agar. As no growth was observed on control plates, these isolates were considered endophytes of plants. Several bacterial species were isolated based on the distinct characteristics of growth. A sum of 280 bacterial isolates were derived from *L. axillare* leaves. Strains with similar morphology and biochemical reactions were grouped into 48 categories. One representative from each category underwent enzymatic and plant growth-promoting (PGP) tests. Identification of these isolates relied on a sequence similarity-based method utilizing the 16S rDNA gene, leading to the identification of forty-eight isolates. The most predominant and diverse genus identified was *Bacillus* constituting 92% of the isolates with 10 different species. The rest of the bacterial isolates belong to *Staphylococcus.* The overall colonization rate of endophytic bacteria that colonize leaf segments was 78.7%.

Morphological and Molecular Characterization

The 16S rDNA region was subjected to PCR amplification employing the forward primer (27 F) and the reverse primer (1492 R), resulting in the generation of an amplicon with an approximate size of 1500 base pairs. These PCR products were subsequently subjected to bidirectional sequencing, utilizing both the forward (27 F) and reverse (1492 R) primers. The resulting nucleotide sequences were aligned to yield fragments ranging from about 1400 to 1500 base pairs in length. These aligned 16S rDNA nucleotide sequences were then deposited into the GenBank database and assigned unique accession numbers. Following BLASTn analysis of the 16S rRNA gene sequences, the endophytic bacterial isolates were identified, and a comprehensive account of these isolates and their closest genetic relatives, based on sequence similarity, is presented in (Table 1). The identified strains were classified into two genera, *Bacillus* and *Staphylococcus*. Our study revealed the presence of 44 isolates from the *Bacillus* genus, encompassing 10 different species (including *Bacillus* sp., *B. pseudomycoides*, *B. cereus*, *B. paramycoides*, *B. velezensis*, *B. subtilis*, *B. thuringiensis*, *B. wiedmannii*, *B. anthracis*, and *B. bacterium*). Additionally, we found that only 4 isolates belong to the *Staphylococcus* genus, covering 3 distinct species (*S. bacterium*, *S. succinus*, and *S. saprophyticus*) (Table 1).

A diverse array of biochemical, physiological, and morphological traits were examined in the initial stages to identify bacterial endophytes. The findings revealed distinctions among the bacterial species isolated from the leaves. The examination of bacterial colonies' morphology revealed a diverse range of colony colors, which included shades of cream, yellow, off-white, and white colors. Subsequent Gram staining analysis revealed that 46 isolates, constituting 95.8% of the total, were characterized as Gram-positive endophytes, whereas 2 isolates, comprising 4.2%, were categorized as Gram-negative.

Biochemical Analysis of the Bacterial Endophytic Isolates

Biochemical tests for bacterial enzymes like protease, lipase, and amylase vary among different bacterial isolates. Out of the entire set of isolates, 42 (87.5%) exhibited lipase activity, with an equal number of 42 (87.5%) demonstrating protease activity. In contrast, only 16 isolates (33.3%) displayed amylase activity (Table 2) (Fig. 2).

Assessment of the Plant Growth-Promoting Features and Halotolerance Capability of the Isolates

All the 48 strains go through in vitro testing to assess seven distinct plant growth-promoting characteristics (Table 3, Fig. 2). Among the 48 isolates, diverse responses were observed in the plant growth-promoting tests, with 47 strains displaying a positive response to at least two of the PGP tests. Forty-five strains (93.8%) exhibited the formation of clearly visible and well-defined clearing zones on Pikovskaya media confirming the potential phosphate solubilization activity. Variations were observed in the biosynthesis of IAA (Indole-3-Acetic Acid) among the strains. A total of 16 strains (33.3%) demonstrated the capability to produce IAA. The CAS agar assay was used to assess the siderophore-producing potential of endophytes. Among the isolates, 44 isolates (91.7%) exhibited the capacity for siderophore production. Twenty-five strains (52.1%) verified the ability to thrive on a DF salt minimal agar medium supplemented with ACC, indicating their possession of ACC deaminase activity. HCN production ability was detected in 20 isolates (41.7%) of the isolates. DNase activity potential was evident in thirteen isolates, accounting for 27.1% of the total. Fifteen isolates (31.3%) were capable of producing ammonia. The salt tolerance of all 48 isolates was evaluated, and the findings revealed that each of them exhibited the ability to withstand of up to (6%) NaCl, while seventeen strains thrived in a medium with a salt concentration of up to (10%) NaCl (Table 3).

Principal Component Analysis (PCA) enabled the identification of potential relationships between variables and the assessed treatments. The primary component (PC1) accounted for the most significant portion of variability in the data, followed by subsequent components (PC2, PC3, etc.) representing the remaining variability. The correlation between the examined bacteria and their plant growth-promoting (PGP) capabilities, which included the production of IAA, siderophore, hydrogen cyanide, NH3, ACC deaminase activity, nitrogen fixation and phosphate solubilization, was analyzed using Principal Component Analysis (PCA) as depicted in (Fig. 3). PC1 explained 31.51% of the total variability, while PC2 accounted for 22.09%. Together, PC1 and PC2 represented 53.6% of the total variability. The graphical biplot demonstrated that the first component (PC1) strongly influenced siderophore production, phosphate solubilization, ammonia production, nitrogen fixation and the ACC deaminase enzyme, whereas PC2 exhibited an influence on the other evaluated characteristics (Fig. 3).

In Vitro Antagonistic Activities Against Fungal Pathogens

The potential antagonistic impact of bacterial endophytic isolates was assessed against various fungal phytopathogens, including *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Fusarium oxysporum*. Antagonistic effectiveness was evaluated in strains that demonstrated a minimum of four positive results in PGP (Plant

Table 1 Molecular identification for bacterial endophytic isolates by 16S rDNA sequencing

S. No.	Name of the bacterial identified	Isolate code	NCBI accession number	Name of the isolates matching with NCBI database and their accession numbers	% Identity
1.	*Bacillus* sp.	L6QU	OQ254813.1	*Bacillus* sp. (OP345219.1)	96
2.	*Bacillus* sp.	L2QU	OQ254812.1	*Bacillus* sp. (OP750263.1)	95
3.	*Bacillus pseudomycoides*	AL17QU	OQ254811.1	*Bacillus pseudomycoides* (MH424709.1)	94
4.	*Bacillus cereus*	AL5QU	OQ254810.1	*Bacillus cereus*(KC999982.1)	99
5.	*Bacillus* sp.	G2QU	OQ254809.1	*Bacillus* sp. (MT521709.1)	92
6.	*Bacillus paramycoides*	L7QU	OQ254808.1	*Bacillus paramycoides* (MT299653.1)	97
7.	*Bacillus* sp.	AL3QU	OQ254807.1	*Bacillus* sp. (KF984428.1)	99
8.	*Bacillus paramycoides*	G15QU	OQ254806.1	*Bacillus paramycoides* (MN793201.1)	99
9.	*Bacillus cereus*	AL24QU	OQ254805.1	*Bacillus cereus*(MN934104.1)	96
10.	*Bacillus* sp.	AL14(1)QU	OQ254804.1	*Bacillus* sp. (KJ601740.1)	97
11.	*Bacillus cereus*	G8QU	OQ254803.1	*Bacillus cereus* (OP747454.1)	98
12.	*Bacillus cereus*	AL19QU	OQ254802.1	*Bacillus cereus* (MG205948.1)	98
13.	*Bacillus cereus*	G13QU	OQ254801.1	*Bacillus cereus* (ON954528.1)	99
14.	*Bacillus velezensis*	AL4QUA	OQ254800.1	*Bacillus velezensis*(OP904217.1)	99
15.	*Bacillus subtilis*	L1qu	OP364396.1	*Bacillus subtilis* (MT626725.1)	100
16.	*Bacillus thuringiensis*	G6qu	OP364395.1	*Bacillus thuringiensis*(OQ726301.1)	99
17.	*Bacillus cereus*	41qu(G16)	OP364386.1	*Bacillus* cereus (MZ292346.1)	99
18.	*Bacillus cereus*	40qu(G14)	OP364385.1	*Bacillus* cereus (OK562654.1)	95
19.	*Bacillus* sp.	38qu (G12)	OP364384.1	*Bacillus* sp. (MK530427.1)	94
20.	*Bacillus* sp.	37qu (G11)	OP364383.1	*Bacillus* sp. (KF496107.1)	94
21.	*Staphylococcaceae bacterium*	36qu (G10)	OP364382.1	*Staphylococcaceae bacterium* (JX064843.1)	97
22.	*Bacillus wiedmannii*	35qu (G9)	OP364381.1	*Bacillus wiedmannii* (MG491522.1)	99
23.	*Staphylococcus succinus*	33qu (G7)	OP364380.1	*Staphylococcus succinus* (MW599383.1)	96
24.	*Bacillus paramycoides*	31qu (G5)	OP364378.1	*Bacillus paramycoides* (OQ566990.1)	99
25.	*Bacillus paramycoides*	29qu (G3)	OP364377.1	*Bacillus paramycoides* (OP420614.1)	99
26.	*Bacillus cereus*	28qu (G1)	OP364376.1	*Bacillus cereus* (MG205955.1)	96
27.	*Bacillus paramycoides*	27qu (AL25)	OP364375.1	*Bacillus paramycoides* (MT903014.1)	99
28.	*Bacillus paramycoides*	26qu (AL22)	OP364374.1	*Bacillus paramycoides* (OQ221523.1)	99
29.	*Bacillus thuringiensis*	25qu (AL21)	OP364373.1	*Bacillus thuringiensis* (MH915619.1)	99
30.	*Bacillus subtilis*	24qu (AL15Y)	OP364372.1	*Bacillus subtilis* (OQ503169.1)	99
31.	*Bacillus thuringiensis*	23qu (AL11)	OP364371.1	*Bacillus thuringiensis* (MH915619.1)	99
32.	*Bacillus cereus*	22qu (AL10)	OP364370.1	*Bacillus cereus* (MH628528.1)	99
33.	Bacillus thuringiensis	21qu (AL9)	OP364369.1	*Bacillus thuringiensis* (OK210309.1)	99
34.	*Bacillus anthracis*	20qu (AL2)	OP364368.1	*Bacillus anthracis* (KT887211.1)	95
35.	*Bacillus thuringiensis*	18qu (AL9W)	OP364366.1	*Bacillus thuringiensis* (MH915619.1)	98
36.	*Bacillus thuringiensis*	17qu (AL14-2)	OP364365.1	*Bacillus thuringiensis* (CP053938.1)	97
37.	*Bacillus cereus*	16qu (AL29)	OP364364.1	*Bacillus cereus* (ON567448.1)	100
38.	*Bacillus cereus*	14qu (AL27)	OP364363.1	*Bacillus cereus* (CP015589.1)	97
39.	*Bacillus thuringiensis*	13qu (AL26)	OP364362.1	*Bacillus thuringiensis* (KR107504.1)	99
40.	*Bacillus paramycoides*	12qu (AL23)	OP364361.1	*Bacillus paramycoides* (OP028080.1)	99
41.	*Bacillus cereus*	11qu (AL20)	OP364360.1	*Bacillus cereus* (MK691597.1)	93
42.	*Bacillus cereus*	9qu (AL18)	OP364359.1	*Bacillus cereus* (ON567448.1)	100
43.	*Staphylococcus saprophyticus*	8qu (AL16)	OP364358.1	*Staphylococcus saprophyticus* (OP804128.1)	93
44.	*Bacillus thuringiensis*	7qu (AL15)	OP364357.1	*Bacillus thuringiensis* (AB244534.2)	98
45.	*Bacillus thuringiensis*	6qu (AL14)	OP364356.1	*Bacillus thuringiensis* (KR107504.1)	97
46.	*Bacillaceae bacterium*	5qu (AL8)	OP364355.1	*Bacillaceae bacterium* (CP045537.1)	100
47.	*Bacillus cereus*	4qu (AL7)	OP364354.1	*Bacillus cereus* (KR997587.1)	95
48.	*Staphylococcus succinus*	1qu (AL1)	OP364353.1	*Staphylococcus succinus* (OP009957.1)	96

Table 2 Hydrolytic enzymes of the bacterial endophytic isolates from leaves of halophyte *L. axillare*

S. No.	Name of the bacterial identified	Isolate code	Extracellular enzyme		
			Lipase	Protease	Amylase
1.	*Bacillus* sp.	L6QU	−	−	+
2.	*Bacillus* sp.	L2QU	−	+	−
3.	*Bacillus pseudomycoides*	AL17QU	+	+	−
4.	*Bacillus cereus*	AL5QU	+	+	+
5.	*Bacillus* sp.	G2QU	+	+	+
6..	*Bacillus paramycoides*	L7QU	−	−	+
7.	*Bacillus* sp.	AL3QU	+	+	−
8.	*Bacillus paramycoides*	G15QU	+	+	+
9.	*Bacillus cereus*	AL24QU	+	+	+
10.	*Bacillus* sp.	AL14(1)QU	−	+	+
11.	*Bacillus cereus*	G8QU	+	+	−
12.	*Bacillus cereus*	AL19QU	+	+	−
13.	*Bacillus cereus*	G13QU	+	+	−
14.	*Bacillus velezensis*	AL4QUA	+	+	+
15.	*Bacillus subtilis*	L1qu	+	+	−
16.	*Bacillus thuringiensis*	G6qu	+	+	−
17.	*Bacillus cereus*	41qu(G16)	+	+	+
18.	*Bacillus cereus*	40qu(G14)	+	+	+
19.	*Bacillus* sp.	38qu (G12)	+	+	+
20.	*Bacillus* sp.	37qu (G11)	+	+	+
21.	*Staphylococcaceae bacterium*	36qu (G10)	+	−	−
22.	*Bacillus wiedmannii*	35qu (G9)	+	+	−
23.	*Staphylococcus succinus*	33qu (G7)	+	−	−
24.	*Bacillus paramycoides*	31qu (G5)	+	+	+
25.	*Bacillus paramycoides*	29qu (G3)	+	+	−
26.	*Bacillus cereus*	28qu (G1)	+	+	−
27.	*Bacillus paramycoides*	27qu (AL25)	+	+	−
28.	*Bacillus paramycoides*	26qu (AL22)	+	+	+
29.	*Bacillus thuringiensis*	25qu (AL21)	+	+	−
30.	*Bacillus subtilis*	24qu (AL15Y)	−	+	+
31.	*Bacillus thuringiensis*	23qu (AL11)	+	+	−
32.	*Bacillus cereus*	22qu (AL10)	+	+	−
33.	*Bacillus thuringiensis*	21qu (AL9)	+	+	−
34.	*Bacillus anthracis*	20qu (AL2)	+	+	+
35.	*Bacillus thuringiensis*	18qu (AL9W)	+	+	−
36.	*Bacillus thuringiensis*	17qu (AL14-2)	+	+	−
37.	*Bacillus cereus*	16qu (AL29)	+	+	−
38.	*Bacillus cereus*	14qu (AL27)	+	+	−
39.	*Bacillus thuringiensis*	13qu (AL26)	+	+	−
40.	*Bacillus paramycoides*	12qu (AL23)	+	+	−
41.	*Bacillus cereus*	11qu (AL20)	+	+	−
42.	*Bacillus cereus*	9qu (AL18)	+	+	−
43.	*Staphylococcus saprophyticus*	8qu (AL16)	−	−	−
44.	*Bacillus thuringiensis*	7qu (AL15)	+	+	−
45.	*Bacillus thuringiensis*	6qu (AL14)	+	+	−
46.	*Bacillaceae bacterium*	5qu (AL8)	+	+	−
47.	*Bacillus cereus*	4qu (AL7)	+	+	−
48.	*Staphylococcus succinus*	1qu (AL1)	+	−	−
	Total positive		42	42	16

Table 2 (continued)

S. No.	Name of the bacterial identified	Isolate code	Extracellular enzyme		
			Lipase	Protease	Amylase
	Percent of positive		87.5	87.5	33.3

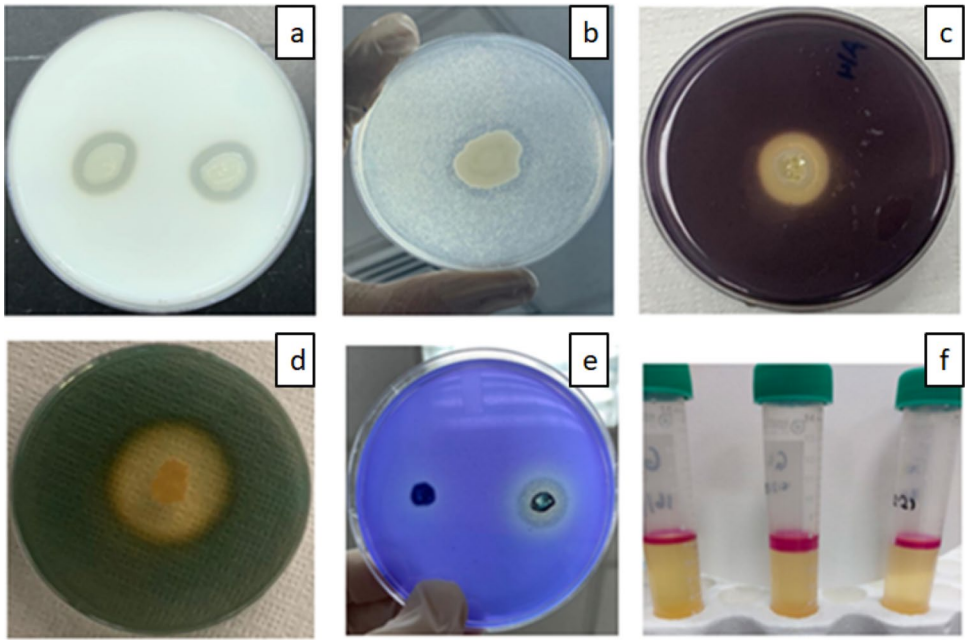

Fig. 2 Graphical representation for some positive biochemical characterization of some bacterial isolates that bosses hydrolysis enzymes and PGP tests: **a** protease, **b** lipase, **c** amylase, **d** siderophore production, **e** phosphate solubilization, and **f** indole acetic acid (IAA)

Growth-Promoting) tests. Consequently, a dual culture assay was conducted for 24 strains only (Table 4).

The bacterial endophytic isolates exhibited distinct responses when confronted with these fungal pathogens. Among them, 13 (54.2%), 15 (62.5%), 16 (66.7%), and 12 (50%) bacterial isolates demonstrated antagonistic behavior against *Botrytis cinerea*, *Alternaria alternata*, *Fusarium oxysporum*, and *Colletotrichum gloeosporioides*, respectively, as observed through the dual culture assay.

Table 4 reveals that only one endophyte out of the 24 isolates obtained from Limonium axillare exhibited inhibitory effects against all four types of pathogens. Specifically, Al4QU displayed remarkable inhibitory activity, with fungistatic effects of 78.0% ± 1.2% against *B. cinerea*, 75.2% ± 0.4 against *A. alternata*, 82.2% ± 0.2 against *F. oxysporum*, and 76.7% ± 0.3 against *C. gloeosporioides*.

Evaluating the Biocontrol and Growth Promotion Effects of Selected Endophyte AL4QUA (*B. velezensis*) on Tomato Seedlings

The strain AL4QUA showed strong in-vitro antagonistic activity against *F. oxysporum* (Fig. 4). Under greenhouse conditions, the strain AL4QUA [(*B. velezensis*) OQ254800.1] demonstrated strong efficacy in controlling tomato seedling growth. The outcomes of the plant growth promotion treatment revealed that strain AL4QUA significantly enhanced the growth of tomato plants within the greenhouse.

Seedlings exposed to the pathogen *F. oxysporum* exhibited adverse effects across all assessed parameters in comparison to other treatment groups. The biomass of these seedlings decreased by nearly 50% (as illustrated in Figs. 5, 6, 7, 8). Based on the figures, the growth and response of the tomato seedlings indicated significant differences ($P \leq 0.05$) in fresh weight, dry weight, shoot length, and chlorophyll content. Conversely, seedlings exposed to both endophytes and the fungus displayed growth patterns comparable to or, in certain parameters, exceeding those of the untreated control group. These results suggest that the endophytes may have a potential positive impact on seedling growth and/or antagonistic properties against the pathogen.

Discussion

The influence of salinity stress, a significant abiotic factor impacting crop growth and yield, particularly in crops like tomato, emphasizes the importance of implementing sustainable agricultural practices (Chebotar et al. 2022; Akram et al. 2019; Verma et al. 2021; Vaishnav et al. 2019). Numerous rhizosphere bacteria known for their plant growth-promoting

Table 3 Plant growth promotor tests for the isolated endophytic bacteria and salinity percentage tolerance for each strain

S. No.	Name of the bacterial identified	Isolate code	Plant growth-promoting potential of isolated bacterial strains							NaCl tolerance range (%)
			IAA	P-solubilization	ACC deaminase activity	Nitrogen fixation	siderophore production	HCN	Ammonia production	
1.	*Bacillus* sp.	L6QU	−	+	−	+	+	−	+	(0–10)
2.	*Bacillus* sp.	L2QU	−	+	−	+	+	+	+	(0–10)
3.	*Bacillus pseudomycoides*	AL17QU	+	+	−	−	+	−	−	(0–6)
4.	*Bacillus cereus*	AL5QU	−	+	+	−	+	−	−	(0–6)
5.	*Bacillus* sp.	G2QU	−	+	−	−	+	−	−	(0–6)
6.	*Bacillus paramycoides*	L7QU	−	+	−	−	+	−	+	(0–10)
7.	*Bacillus* sp.	AL3QU	−	+	+	−	+	−	-	(0–10)
8.	*Bacillus paramycoides*	G15QU	+	+	−	−	+	−	-	(0–6)
9.	*Bacillus cereus*	AL24QU	−	+	+	+	+	−	+	(0–10)
10.	*Bacillus* sp.	AL14(1)QU	−	−	−	−	+	+	−	(0–6)
11.	*Bacillus cereus*	G8QU	−	+	+	+	+	−	+	(0–6)
12.	*Bacillus cereus*	AL19QU	−	+	−	−	+	−	−	(0–10)
13.	*Bacillus cereus*	G13QU	−	+	+	−	+	−	−	(0–6)
14.	*Bacillus velezensis*	AL4QUA	+	+	+	+	+	+	+	(0–10)
15.	*Bacillus subtilis*	L1qu	−	+	−	−	+	−	−	(0–10)
16.	*Bacillus thuringiensis*	G6qu	−	+	+	+	+	+	+	(0–10)
17.	*Bacillus cereus*	41qu(G16)	+	+	+	+	+	−	+	(0–6)
18.	*Bacillus cereus*	40qu(G14)	−	+	−	−	+	−	−	(0–6)
19.	*Bacillus* sp.	38qu (G12)	+	+	−	−	+	+	−	(0–6)
20.	*Bacillus* sp.	37qu (G11)	+	+	−	+	+	−	+	(0–6)
21.	*Staphylococcaceae bacterium*	36qu (G10)	−	+	+	−	+	+	−	(0–6)
22.	*Bacillus wiedmannii*	35qu (G9)	+	+	+	+	+	+	+	(0–10)
23.	*Staphylococcus succinus*	33qu (G7)	−	+	−	−	+	−	−	(0–10)
24.	*Bacillus paramycoides*	31qu (G5)	+	+	−	−	+	+	−	(0–6)
25.	*Bacillus paramycoides*	29qu (G3)	+	+	+	−	−	−	−	(0–6)
26.	*Bacillus cereus*	28qu (G1)	+	+	+	−	+	+	−	(0–6)
27.	*Bacillus paramycoides*	27qu (AL25)	−	+	−	−	+	−	−	(0–6)
28.	*Bacillus paramycoides*	26qu (AL22)	−	+	−	−	+	+	−	(0–10)
29.	*Bacillus thuringiensis*	25qu (AL21)	−	+	+	+	+	+	+	(0–10)
30.	*Bacillus subtilis*	24qu (AL15Y)	−	+	−	−	+	+	−	(0–6)
31.	*Bacillus thuringiensis*	23qu (AL11)	+	+	−	−	+	+	−	(0–6)
32.	*Bacillus cereus*	22qu (AL10)	+	+	−	−	+	+	−	(0–6)
33.	*Bacillus thuringiensis*	21qu (AL9)	−	+	−	−	+	−	+	(0–6)
34.	*Bacillus anthracis*	20qu (AL2)	+	+	−	−	+	+	−	(0–6)
35.	*Bacillus thuringiensis*	18qu (AL9W)	−	+	+	−	−	−	−	(0–6)
36.	*Bacillus thuringiensis*	17qu (AL14-2)	−	−	−	−	−	−	−	(0–6)
37.	*Bacillus cereus*	16qu (AL29)	−	+	+	−	+	+	−	(0–6)

Table 3 (continued)

S. No.	Name of the bacterial identified	Isolate code	Plant growth-promoting potential of isolated bacterial strains						NaCl tolerance range (%)
			IAA	P-solubilization	ACC deaminase activity	Nitrogen fixation	siderophore production	HCN	Ammonia production	
38.	*Bacillus cereus*	14qu (AL27)	–	+	+	-	+	–	–	(0–6)
39.	*Bacillus thuringiensis*	13qu (AL26)	–	+	+	+	+	–	+	(0–10)
40.	*Bacillus paramycoides*	12qu (AL23)	–	+	+	-	+	–	–	(0–10)
41.	*Bacillus cereus*	11qu (AL20)	–	+	+	+	+	–	+	(0–6)
42.	*Bacillus cereus*	9qu (AL18)	–	+	+	+	+	–	+	(0–10)
43.	*Staphylococcus saprophyticus*	8qu (AL16)	–	+	–	–	+	–	–	(0–6)
44.	*Bacillus thuringiensis*	7qu (AL15)	+	+	+	–	+	+	–	(0–6)
45.	*Bacillus thuringiensis*	6qu (AL14)	–	–	+	–	–	+	–	(0–6)
46.	*Bacillaceae bacterium*	5qu (AL8)	+	+	+	–	+	+	–	(0–10)
47.	*Bacillus cereus*	4qu (AL7)	–	+	+	–	+	–	–	(0–6)
48.	*Staphylococcus succinus*	1qu (AL1)	+	+	+	–	+	+	–	(0–6)
	Total positive		16	45	25	13	44	20	15	17 Strains more than 8%
	Percent of positive		33.3	93.8	52.1	27.1	91.7	41.7	31.3	35.4

abilities have the capacity to alleviate the adverse impacts of abiotic stressors, such as salinity and heavy metals, through a range of direct and indirect mechanisms (Gkorezis et al. 2016; Albureikan 2023). These beneficial bacteria ultimately contribute to improved crop development and increased yields (Albureikan 2023). Isolating, identifying, and examination of endophytes derived from halophytes are as important as the examination of rhizosphere bacteria that inhibit the halophytes. In the current study, we have undertaken an investigation into the diverse bacterial endophytes present within the leaf tissues of the intriguing halophyte *Limonium axillare*, which thrives in arid and semi-arid environments. Our research explores these endophytes' potential for promoting plant growth and their capacity for exhibiting antagonistic properties. To the best of our knowledge, this marks the initial isolation and examination of endophytes from *Limonium axillare*. Out of the 48 bacterial endophytes identified in this study, it was evident that Gram-positive bacteria, specifically, those belonging to the genus *Bacillus*, were the most prevalent. Our findings are consistent with prior research on the isolation of bacterial endophytes from *Suaeda heteroptera*, where *Bacillus* was similarly identified as the predominant genus of endophytic bacteria, accounting for 30.4% of the total isolates (Niu et al. 2011). Earlier research has consistently highlighted the prevalence of *Bacillus* spp. in diverse agro-climatic regions and environments subject to natural challenges (Misra and Chauhan 2020; Mokrani et al. 2020; Semwal et al. 2023; Misra et al. 2017). Furthermore, the outcomes of the 16S rRNA gene sequencing are consistent with recent reports that underscore the prominent role of *Bacillus* spp. as a crucial element in the plant endosphere (Cochard et al. 2022; Semwal et al. 2023; Christakis et al. 2021). The dominance of the *Bacillus* genus can be attributed to its greater adaptability to saline environments, as supported by studies (Sgroy et al. 2009; Yaish et al. 2015; Singh et al. 2021; Glick 2014).

In our research, all 48 isolates exhibited growth in the presence of 6% NaCl, while only 17 strains demonstrated growth in the presence of 10% NaCl. These strains hold great potential as candidates for the creation of bioinoculants, which could play a pivotal role in supporting salt soil phytoremediation efforts and alleviating salt-induced stress. Our findings align with earlier research focused on the isolation of endophytic bacteria from three halophytic plants, namely *Cakile maritima*, *Matthiola tricuspidata*, and *Crithmum maritimum* (Christakis et al. 2021). That study revealed that a substantial portion of their isolates exhibited growth tolerance at elevated salt concentrations ranging from 5 to 17% NaCl (Christakis et al. 2021). Furthermore, the results from both endophytic and rhizospheric bacteria isolated from *Arthrocnemum macrostachyum* demonstrated remarkable tolerance to salt levels of up to 10% NaCl (Khan et al. 2022).

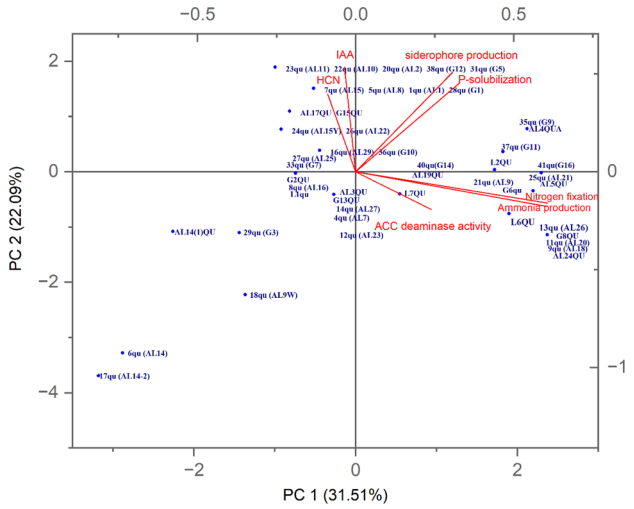

Fig. 3 Principal component analysis (PCA) showing the relationships between the examined bacterial endophytic strains and their (PGP) characteristics

Plant growth-promoting bacteria (PGPB) have a crucial role in governing soil fertility, facilitating nutrient cycling, and enhancing plant growth. PGPB are well-known for their significant capability in secreting phytohormones such as indole-3-acetic acid (IAA), hydrolytic enzymes like (protease, lipase and amylase), production of various metabolites [siderophore, hydrogen cyanide (HCN), 1 aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase)], ammonia production and fixation of nitrogen (Lahlali et al. 2022; Tiwari et al. 2019; Ajijah et al. 2023). These compounds play a crucial role in enhancing plant growth and bolstering resistance to stress. Increasing nutrient availability in the soil by nitrogen fixation, phosphate solubilization and chelating iron by siderophore production. In this current study, the bacterial isolates exhibited significant potential in the production of various plant growth-promoting substances as reported in Table 3. Approximately (33.3%) of the bacterial isolates were capable of producing IAA, (52.1%) exhibited ACC deaminase activity, and a substantial (91.75%) demonstrated the ability to produce siderophores. These findings align with recent research that isolated multiple endophytic bacterial strains associated with halophytes, many of which exhibited unique plant growth-promoting traits; these findings demonstrate comprehensive enhancements in plant growth under saline stress (Sahu et al. 2023; Barcia-Piedras et al. 2023; Zhang et al. 2019; Kang et al. 2019). One of the primary plant growth-promoting

Table 4 Antagonistic activities of bacterial endophytes against fungal phytopathogens (Means ± S.D)

S. No.	Bacterial strain	*Botrytis cinerea*	*Alternaria alternata*	*Fusarium oxysporum*	*Colletotrichum gloeosporioides*
1.	L6QU	00.0 ± 0.0	46.3 ± 1.5	71.6 ± 0.5	00.0 ± 0.0
2.	L2QU	75.2 ± 0.4	72.2 ± 0.2	51.4 ± 0.4	00.0 ± 0.0
3.	AL24QU	00.0 ± 0.0	41.1 ± 0.6	00.0 ± 0.0	75.2 ± 0.4
4.	G8QU	57.7 ± 0.8	00.0 ± 0.0	65.2 ± 0.5	00.0 ± 0.0
5.	AL4QUA	78.0 ± 1.2	75.2 ± 0.4	82.2 ± 0.2	76.7 ± 0.3
6.	G6qu	66.3 ± 0.8	00.0 ± 0.0	46.1 ± 0.3	00.0 ± 0.0
7.	41qu(G16)	00.0 ± 0.0	67.7 ± 0.3	00.0 ± 0.0	66.3 ± 0.8
8.	38qu (G12)	27.4 ± 0.9	00.0 ± 0.0	61.2 ± 0.4	57.2 ± 0.2
9.	37qu (G11)	44.5 ± 0.4	66.3 ± 0.8	00.0 ± 0.0	00.0 ± 0.0
10.	36qu (G10)	00.0 ± 0.0	57.2 ± 0.2	77.7 ± 0.3	00.0 ± 0.0
11.	35qu (G9)	00.0 ± 0.0	37.4 ± 0.1	00.0 ± 0.0	00.0 ± 0.0
12.	31qu (G5)	37.1 ± 0.7	44.5 ± 0.4	56.5 ± 0.4	00.0 ± 0.0
13.	28qu (G1)	24.5 ± 0.6	00.0 ± 0.0	48.9 ± 07	37.4 ± 0.1
14.	25qu (AL21)	00.0 ± 0.0	00.0 ± 0.0	67.1 ± 0.6	44.5 ± 0.4
15.	23qu (AL11)	00.0 ± 0.0	00.0 ± 0.0	54.5 ± 0.3	00.0 ± 0.0
16.	22qu (AL10)	61.9 ± 0.1	30.5 ± 0.6	00.0 ± 0.0	00.0 ± 0.0
17.	20qu (AL2)	45.8 ± 1.8	00.0 ± 0.0	00.0 ± 0.0	69.3 ± 0.4
18.	16qu (AL29)	39.7 ± 0.9	00.0 ± 0.0	00.0 ± 0.0	61.3 ± 0.9
19.	13qu (AL26)	00.0 ± 0.0	69.3 ± 0.4	20.3 ± 0.7	39.7 ± 0.9
20.	11qu (AL20)	61.1 ± 0.4	61.3 ± 0.9	78.0 ± 1.2	00.0 ± 0.0
21.	9qu (AL18)	54.5 ± 0.3	39.7 ± 0.9	38.1 ± 0.9	00.0 ± 0.0
22.	7qu (AL15)	00.0 ± 0.0	00.0 ± 0.0	29.4 ± 1.4	56.5 ± 0.7
23.	5qu (AL8)	00.0 ± 0.0	51.1 ± 0.5	00.0 ± 0.0	43.4 ± 0.7
24.	1qu (AL1)	00.0 ± 0.0	64.8 ± 1.5	57.7 ± 0.5	57.2 ± 0.2

Fig. 4 Dual culture assay for the endopyhtic bacterial isolate (AL4QUA) against fungal phytopathogen *F. oxysporum*

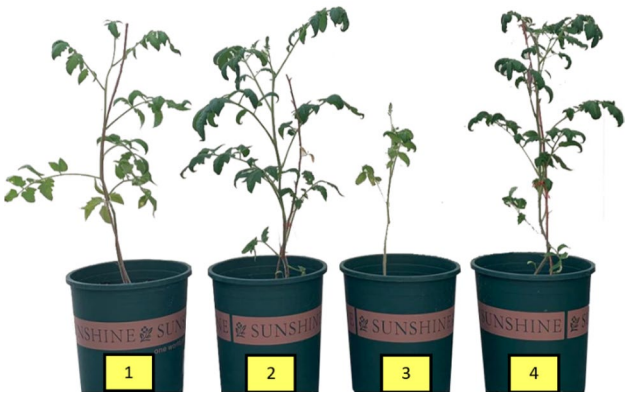

Fig. 5 A photo of tomato seedlings after 6 weeks of treatment. (**1**) Untreated control, (**2**) seedlings inoculated with endophyte strain (AL4QUA), (**3**) seedlings inoculated with the pathogen *Fusarium oxisporum* spore suspension. (**4**) seedlings inoculated first (1 week earlier) with endophytes (AL4QUA) and then inoculated with the plant pathogen *F. oxisporum*

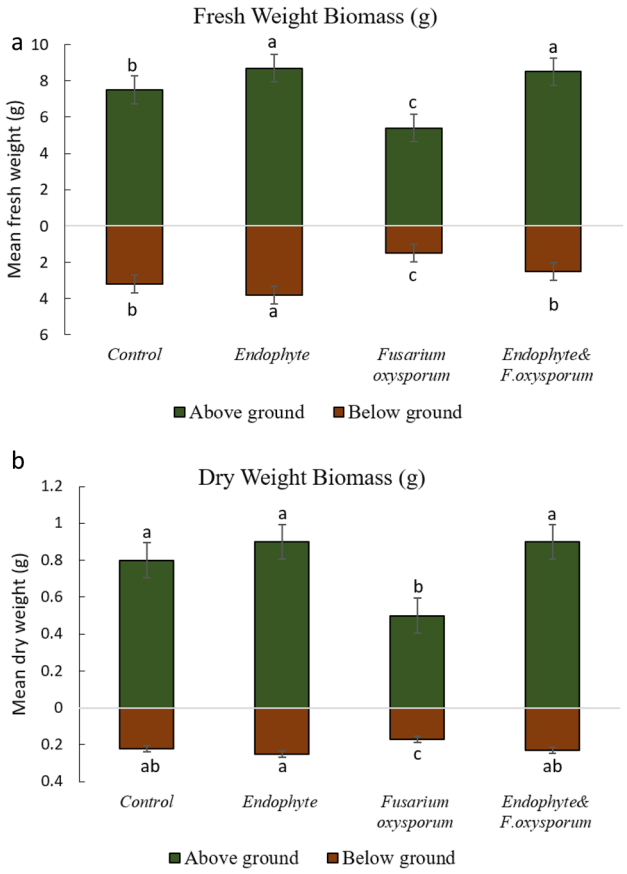

Fig. 6 Effect of endophyte and pathogen treatments on biomas of belowground and aboveground biomass of tomato seedlings after 6-weeks of treatment. Graph **A** represent the fresh weight biomass for above and below ground. Graph **B** represent the dry weight biomass for above and below ground. Error bars refer to standard errors of the means. Values that share common letter(s) are considered not significant at $p \leq 0.05$ according to Tukey's test ($N=4$)

(PGP) mechanisms involves ACC deaminase, which mitigates the impact of salt stress by reducing ethylene levels in plants (Dragojević et al. 2023; Madhaiyan et al. 2006; Glick 2014). While salt stress typically inhibits the synthesis of indole-3-acetic acid (IAA) in plants, endophytic bacteria can compensate by producing their own IAA, promoting the growth of plant roots, stems, and leaves(Selvakumar et al. 2016; Choudhury et al. 2021). Another mechanism entails the production of siderophores, which not only dissolve soil iron for plant uptake but also deter plant pathogens (Christakis et al. 2021; Sorty et al. 2016; Zhou et al. 2017).

Furthermore, *Limonium axillare* harbored a wealth of endophytic bacteria possessing N2-fixation (27.1%) and phosphate-solubilization (93.8%) capabilities. The presence of such bacteria likely contributes to the ability of *Limonium axillare* to thrive in nutrient-poor soil, particularly in conditions with limited nitrogen and phosphorus availability.

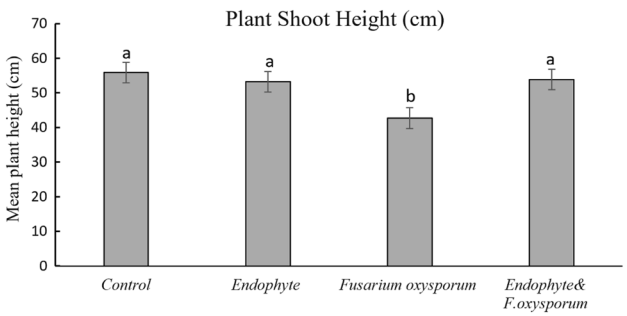

Fig. 7 Effect of endophyte and pathogen treatments on shoot lenght of tomato seedlings after 6-weeks of treatment. Error bars refer to standard errors of the means. Values that share common letter(s) are considered not significant at $p \leq 0.05$ according to Tukey's test ($N=4$)

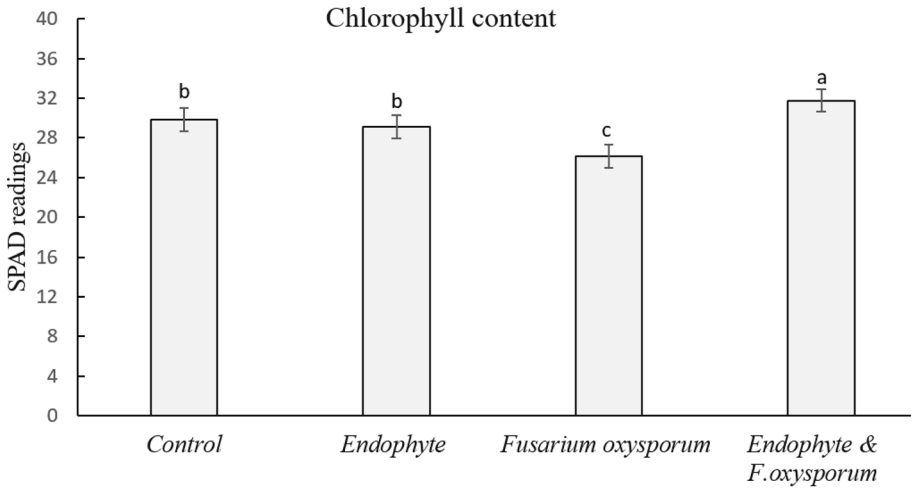

Fig. 8 Effect of endophyte and pathogen treatments on chlorophyll content of leaves of tomato seedlings after 6-weeks of treatment. Error bars refer to standard errors of the means. Values that share common letter(s) are considered not significant at $p \leq 0.05$ according to Tukey's test ($N = 4$)

The adverse impacts associated with chemical pesticides, such as phytotoxicity, pathogen resistance, and chemical residues, have ignited significant interest in the development of novel antimicrobial agents (Sorty et al. 2016; Shahzad et al. 2017). Endophytic bacteria have emerged as promising biocontrol alternatives due to their capacity to produce antimicrobial compounds, the presence of siderophores, and their ability to induce systemic resistance against plant diseases(Khan et al. 2018; Shahzad et al. 2017; Chen et al. 2021).

In the present study, the antimicrobial potential of a selected group of bacterial isolates (24 isolates) displaying at least four plant growth-promoting (PGP) traits was evaluated against four plant pathogens. Impressively, nearly all 24 strains exhibited substantial antimicrobial activity against two or more of the tested pathogens (as depicted in Table 4).

Bacillus species have been extensively researched for their potential as microbial biocontrol agents (Tian et al. 2020; Das et al. 2014; Beneduzi et al. 2012). These beneficial microorganisms, when applied in agriculture, can influence plant development and fruit production by enhancing nutrient uptake and engaging in a range of enzyme activities that counteract pathogenic bacteria and fungi (Fonseca et al. 2022; Beneduzi et al. 2012). The dual culture assay has proven to be an effective and valued qualitative method for the assessment of microorganisms' antagonistic activities against fungal phytopathogens. We conducted a search for indigenous bacteria found in *L.axillare* that exhibited the potential to exert antagonistic effects on the four primary fungi responsible for crop losses, *A. alternate*, *B. cinerea*, *C. gloeosporioides* and *F. oxysporum*. Notably, recent reports have expanded our understanding of the disease-controlling capabilities of *B. velezensis*, underscoring their significance as biocontrol agents. For instance, Kim et al. (2021) highlighted the effectiveness of *B. velezensis* (AK-0) against *C. gloeosporoides*, the causal agent of apple bitter rot. Additionally, Palazzini et al. (2016) demonstrated, through greenhouse and field trials, that *B. velezensis* (RC 218) could reduce the severity of *Fusarium* head blight and the associated mycotoxin deoxynivalenol. Furthermore, *B. velezensis* (NKG2) exhibited in vitro antagonistic effects against several major fungal plant pathogens, including *B. cinerea*, *A. alternata*, *Fusarium graminareum*, *Fusarium oxysporum*, *Fulvia fulva*, and *Ustilaginosa virens* (Myo et al. 2019). Moreover, Chacón et al. (2022) isolated epiphytic bacteria *Bacillus velezensis* (BA3 and BA4) from blueberry and showed their antifungal activity against *B. cinerea* and *A. alternate*. The isolation of *B. velezensis* (TSA32-1) from the soil revealed its ability to exert antimicrobial activity against phytopathogenic fungi, manifested as the inhibition of *Fusarium graminearum*, *F. fujikuroi*, *Alternatia alternate*, and *Diaporthe actinidiae* growth (Kim et al. 2022).

Diseases pose a significant constraint on tomato cultivation, with fungal diseases being particularly troublesome. *Fusarium oxysporum*, a soil-borne hemibiotrophic fungal pathogen, significantly hampers tomato crop yields in both greenhouse and open-field environments. In the advanced stages of infection, this fungal pathogen infiltrates the xylem vessels, leading to a gradual wilting and, ultimately, the demise of the plant (Agrios 2005). *F. oxysporum* is a widely recognized plant ailment that inflicts substantial damage on a range of crops, both in the field and during postharvest storage global tomato production varies from year to year but consistently ranks among the top vegetables produced worldwide. In 2020, the global production of tomatoes was approximately 182 million metric tons, making it one of the most widely grown and consumed vegetables. Biocides have traditionally been employed as chemical means to manage soil-borne pathogens. Nevertheless, this approach poses environmental risks, and certain chemicals are no longer in use. Host resistance and the utilization of biocontrol agents are now supplanting these conventional methods of pest

control (Herrera-Téllez et al. 2019). The results indicated that the endophytes might enhanced the growth of the seedlings and/or antagonize the pathogen. There are two mechanisms for endophyte to promote plant growth either directly or indirectly. Direct mechanism involves in the promoting of the plant growth by facilitating the gaining of required nutrients and by modulating the level of hormones within the plant. Such essential nutrients include phosphorus, iron and nitrogen (Santoyo et al. 2016). Endophytes either can involve in synthesizing several phytohormones such as cytokinin, gibberellin and auxin, or may involve in lowering the level of ethylene by synthesizing (ACC) enzyme that responsible for cleaves of the immediate precursor of ethylene (ACC) compounds (Hardoim et al. 2015). For the indirect promotion of the growth of plant, endophytes involve in inhibition of pathogens and parasites that caused plant diseases such as fungi, bacteria (Saini et al. 2015), insects and nematodes (Santoyo et al. 2016).

Conclusion

In summary, the *B. velezensis* (AL4QUA) strain, isolated from the halophyte *Limonium axillare*, exhibits promising potential for the effective management of phytopathogenic fungi. This positions it as a strong candidate for use as a biological control agent in the cultivation of local crops. Our findings not only corroborate previous reports on the in vitro bioactivities of this species but also mark a significant achievement in isolating antifungal *B. velezensis* strains from *L. axillare* and evaluating their effectiveness against fungal pathogens in both in vitro and in vivo settings. Future research will prioritize uncovering the mechanisms that underlie biological control, including the exploration of bioactive compounds. Additionally, complete genome sequencing of these potential agents will contribute to a better understanding of the genetic and physiological mechanisms driving their control over phytopathogenic fungi.

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Author Contributions FA and MHA designed the study, FA and ZB performed the experiments, FA, MHA, analyzed the results, FA wrote the manuscript. MHA edited the manuscript. MHA reviewed the manuscript and MHA supervised the work.

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Data Availability All data sets produced or examined in the current study are incorporated within this manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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