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## Isolation and identification of endophytic bacteria from *Mentha longifolia* and their application for the enhancement of wheat growth under salt conditions

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Various plant growth-promoting endophytic bacteria (PGPE) have been shown in numerous publications to help their host plants adapt to a variety of biotic and abiotic challenges. This is advantageous when attempting to strengthen protection against these stressors and increase plant productivity. Chemical pesticides and fertilizers have been replaced by endophytic bacteria. The purpose of this work was to isolate endophytic bacteria (EB) from Mentha longifolia and screen the bacterial processes involved in promoting plant growth. Three out of ten isolates were selected for further analysis based on attributes such as nitrogen fixation, phosphate-solubilizing activities, production of indole-3-acetic acid and ammonia, and salt tolerance. The 16S rRNA gene sequencing analysis revealed that these isolates belong to Streptomyces mutabilis, Priestia megaterium, and Bacillus pumilus. The plant-promoting properties were evaluated, and their effects on the early stages and vegetative growth of wheat (Triticum aestivum L.) were observed using the paper towel method and pot tests. Compared to the non-inoculated control, the PGPE treatment frequently showed a significant increase in germination percentage, root and shoot length, and other growth parameters of wheat. These effects were particularly noticeable on plant growth under salt stress. Based on these findings, it is possible to use B. pumilus, P. megaterium, and S. mutabilis as biofertilizers to help T. aestivum cope with salt stress.

**Keywords:** Endophytic bacteria; PGPB; 16S rRNA gene sequencing; salinity stress; *Triticum aestivum L*; Germination

### INTRODUCTION

Agriculture is impacted by abiotic factors such as salinity, temperature, and drought. According to Gupta and Pandey (2019), Jabborova et al. (2021), and Kapadia et al. (2021), these are the primary challenges to sustainable agriculture worldwide. Salinity is one of the most well-known factors affecting agricultural productivity, influencing plant physiological development, seedling germination, and final crop production (Egamberdieva, 2009; Budran et al., 2023; Taha et al., 2023). Semi-arid or dry areas are more prone to salinization due to reduced freshwater availability for dissolving salts and higher evaporation rates (Fazeli-Nasab & Sayyed, 2019). Currently, approximately 20% of the cultivated land area and 33% of irrigated agricultural land are affected by high salinity (Shrivastava & Kumar, 2015). By 2050, the salinization rate of cultivated land is expected to exceed 50% (Singh, 2022). One way to mitigate the environmental stress that soil salts place on plants is to use beneficial microorganisms as biofertilizers, rather than chemical fertilizers or pesticides (Ahmed et al., 2021; Nehl et al., 1997). It is believed that endophytes, which have adapted to their environments, coexist symbiotically with all plants in natural ecosystems. These endophytes can significantly influence the host plants' ability to adapt and tolerate stress (Redman et al., 2011; Rodriguez et al., 2008).

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Plant-growth-promoting endophytes (PGPE) are beneficial microorganisms that inhabit plant tissues, antagonize certain plant pathogens, and promote the growth and development of host plants (Zhang et al., 2022). PGPE bacteria offer various benefits to the plants they colonize, including growth promotion, metabolic modulation, and phytohormone signaling that enhances adaptability to biotic and abiotic stresses (Eid et al., 2021; Lata et al., 2018). PGPE has been shown to improve plants' resistance to salinity stress through various mechanisms (Hashem et al., 2015; Numan et al., 2018; Yaish et al., 2015). The stress alleviation mediated by PGPE in the host occurs through two mechanisms: activation of the host response systems and biosynthesis of anti-stress compounds, such as enzymatic and non-enzymatic antioxidants and phytohormones (Aizaz et al., 2023). Several crops have demonstrated improved salt tolerance when exposed to certain PGPR, including Rhizobium, Pseudomonas, Azospirillum, Arthrobacter, Flavobacterium, and Bacillus (Almaghrabi et al., 2014).

Mentha is an important medicinal plant cultivated worldwide. It belongs to the Lamiaceae family, which comprises about 42 species (Alreedy, 2022). Mentha grows naturally in Africa, Asia, Europe, Australia, and North America (Salehi et al., 2018). Several species, including Mentha arvensis L. (cornmint), Mentha citrate Ehrh. (bergamot mint), Mentha x piperita, Mentha spicata L., and Mentha longifolia L., are renowned for their culinary, medicinal, and aromatic properties. They are particularly known for their benefits in gastrointestinal, respiratory, infectious, and inflammatory conditions (Farzaei et al., 2017). Horse mint (*M. longifolia L.*), a valuable member of the mint genus, has shown potential for its medicinal properties. Studies suggest it may benefit the digestive and nervous systems and may possess anticancer and antioxidant effects (Abbas & Nisar, 2020; Patti et al., 2020). *M. longifolia L.* is considered one of the most promising sources of biologically active substances for the food, cosmetics, and pharmaceutical industries (Hudz et al., 2023).

*Triticum aestivum L.*, or wheat, provides approximately 20 percent of the calories consumed each day and is a major source of energy for humans. It contains essential nutrients such as protein, vitamins, and phytochemicals (Chaves et al., 2013). Worldwide, wheat is a significant staple crop, but salt stress reduces its nutritional value and productivity (Miransari & Smith, 2019). According to a study by Tester and Langridge (2010), there is a need to increase wheat production by at least 50% due to the projected 60% rise in wheat demand by the year 2050. In Egypt, specifically, this gap has been steadily widening and has recently reached approximately 55% (Abulela et al., 2022).

The objective of the current study was to isolate, characterize, and select endophytic bacteria from *Mentha longifolia* that exhibit high resistance to salt and possess plant-growth-promoting (PGP) characteristics. The isolates were identified and phylogenetically described using 16S rRNA gene analysis. Finally, we examined how these PGP endophytic bacteria (PGPEB) affect wheat (*Triticum aestivum L.*) plants under salt stress, leading to enhanced seed germination, root growth, stem length, and overall vegetative growth.

### **MATERIALS AND METHODS**

## Plant collection and endophytic bacteria (EB) isolation

The collection of *Mentha longifolia* plant samples was conducted from barren soils affected by salt in the Beni Suef governorate. The collected samples were carefully washed under running tap water for 10 minutes to remove any adhering soil particles. Surface sterilization was performed following the procedure described by AlKahtani et al. (2020). To

isolate the endophytic bacteria (EB), 1 gram of the surface-sterilized plant tissue was placed in a sterile mortar and macerated in 9 milliliters of phosphatebuffered saline (PBS). One milliliter of the tissue extract was then distributed among various isolation media using serial dilution. The plates were incubated for a week at 30°C, during which the formation of bacterial colonies was observed. Each bacterial isolate was preserved in 25% glycerol and stored at -80°C.

## Screening isolated endophytic bacteria for plant growth promotion (PGP) activities

**Indole Acetic Acid (IAA) assay:** To evaluate their ability to produce IAA, EB isolates were cultured in ISP2 broth supplemented with 0.1% L-tryptophan and incubated in a shaking incubator at  $30 \pm 2$  °C with a rotation speed of 120 rpm for 3-4 days. The resulting pink color was measured using a UV spectrophotometer at 530 nm, and the IAA concentration was determined against a standard curve (Bric et al., 1991; Shahid & Khan, 2018).

**Phosphate Solubilization Assay:** We evaluated the phosphate solubilizing abilities of bacterial isolates using a method established by Surange et al. (1997). This method involves Pikovskaya agar, a specialized medium that contains nutrients and bromophenol blue as a pH indicator. After incubating the inoculated plates for 48 hours at 30°C, the presence of a yellow halo surrounding the bacterial colonies indicated the isolates' capacity to solubilize phosphate.

**Nitrogen Fixation Activity:** We investigated the nitrogen fixation abilities of bacterial isolates using two nitrogen-free growth media: Ashby's mannitol agar and NFC medium. The isolates were incubated on these media for seven days at a constant temperature of 28°C. Colony formation was then used as an indicator of the isolates' capacity to fix nitrogen (Liu et al., 2016; Li et al., 2018).

Ammonia Production: The evaluation of ammonia (NH<sub>3</sub>) production by isolated endophytic bacterial strains was conducted by incubating these strains in peptone water. The composition of the peptone water included 10 g/L of peptone, 5 g/L of NaCl, and 1 L of distilled H<sub>2</sub>O. The incubation lasted for 72 hours at a temperature of  $35 \pm 2$  °C. A control was established using peptone water without any bacterial strains. To measure ammonia production, 1 mL of Nessler's reagent was added to the peptone liquid medium. The presence of ammonia was

indicated by a color shift: a faint yellow color signified minimal production, while a deep yellow to brownish hue indicated maximal production (Singh et al., 2014).

## Salt tolerance assay for the isolated endophytic bacteria

The assessment of the bacterial isolates' natural resistance to salinity involved monitoring their growth on ISP2 medium supplemented with various NaCl concentrations, specifically 2.5%, 5%, 7.5%, and 10% (w/v). The medium was incubated for one week at 30  $\pm$  2°C to observe the salt-tolerant strains (Ramadoss et al., 2013).

## Phylogenetic and identification of the selected endophytic bacteria

Sequence analysis and identification were performed using the BigDye<sup>™</sup> Terminator v3.1 Cycle Sequencing Kit (ABI Applied Biosystems) from Microgen in Korea. The extraction of genomic DNA from strains and PCR amplification of the 16S rRNA gene were carried out as described by Hesham (2014). The 16S rRNA gene was amplified using bacterial universal primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT). The 16S rDNA sequences of bacterial isolates were compared with sequences available in GenBank using BLASTN with non-redundant (nr) and microbial databases (Mawad et al., 2016). Phylogenetic trees of 16S rDNA sequences of bacterial isolates were constructed against reference bacterial sequences identified in BLAST searches using MEGA 11 software v11.5 (Tamura et al., 2013). The 16S rRNA gene sequences of all isolates were deposited in the GenBank database under accession numbers PP496558 (BSU-E6), PP496559 (BSU-E8), and PP496560 (BSU-E23). Phylogenetic trees were constructed as described by Hesham et al. (2020).

## Wheat seeds inoculate with the selected endophytic bacteria

Mature seeds were collected from Misr 3 (T. aestivum L) wheat plants growing in a single field. Surface sterilization was performed as described by Lastochkina et al. (2017). Pure cultures of selected bacterial isolates were grown in ISP2 broth at 30 °C and diluted to a final concentration of 10^6 CFU ml^-1 in sterile distilled water. The seeds were then co-cultured with bacterial isolates. The seeds were mixed with the bacterial suspension on a rotary shaker at 120 rpm in a 50 mL flask for 2 hours at

room temperature. Control seeds were treated with sterile distilled water without bacterial culture.

## Wheat seed germination under different salt concentrations

Using sterile sieves, the seeds were extracted from the bacterial suspension. According to Jha et al. (2012), thirty seeds were germinated in petri dishes lined with two layers of sterile filter paper for each treatment. Dishes were wet with 100, 200, and 300 mM NaCl solutions. All germination studies were conducted with three replicates for each condition. The number of seeds that germinated was counted after the seeds had grown for five days at 25°C in the dark. The percentage of germination was indicated by the number of seeds that produced the shortest rootlet length (Mokronosova, 1994).

# Effect of inoculation with selected endophytic bacteria on weight seedling growth under different salt concentrations

A bacterial suspension was prepared and applied to sterilized seeds for two hours. Parallel controls were maintained by cultivating wheat seeds without inoculation of endophytes. Seeds were planted in plastic containers filled with a 1:1:1 volume ratio of soil, peat moss, and sand, with five seeds placed in each pot. The following week, 10 mL of bacterial suspension containing 1x10<sup>6</sup> CFU/mL was injected at the root zone, and the pots were irrigated with a saline solution (100, 200, and 300 mM NaCl) approximately every five days. Each treatment included three pots, and each pot contained five wheat plants. After 40 days, the plants were removed from the growth container, and the length of the roots and shoots, as well as the dry and fresh weights, were measured to assess the growthpromoting effects.

### Statistical analysis

Three biological replicates were used for each microbiological test, as well as for tests on seed germination and wheat development metrics. The data were presented as mean  $\pm$  SD (standard deviation). Statistical significance was assessed using the Analysis of Variance (ANOVA) tool in Microsoft Excel 2010.

### RESULTS

## Isolation and screening of endophytic bacteria PGP activities

In the study, ten endophytic bacterial strains (BSU-E1, BSU-E3, BSU-E6, BSU-E7, BSU-E8, BSU-E13, BSU- E16, BSU-E18, BSU-E22, and BSU-E23) were isolated from the \*M. longifolia\* plant. Six bacterial strains were isolated from the root, while the remaining four were isolated from the shoot tip. Each strain underwent in vitro testing for several characteristics that promote plant growth, as shown in Figure 1 and Table 1. The findings revealed that seven isolates were positive for IAA production. Isolate BSU-E6 exhibited the highest IAA activity (58.57 µg/mL), whereas the lowest activity was recorded for strain BSU-E16 (8.68 µg/mL) (Table 1). Additionally, three of the ten isolates (BSU-E6, BSU-E8, and BSU-E23) demonstrated the ability to solubilize phosphate, as evidenced by distinct zones surrounding their colonies on Pikovskaya's agar plate. Nitrogenase activity was observed in Ashby's free nitrogen medium and NFC for seven isolates, suggesting that the bacterial endophytes can fix nitrogen from the atmosphere. Conversely, ammonia production was noted in 8 out of 10 bacterial isolates, as indicated by the color change in the growth media after adding Nessler's reagent (Table 1). According to Table 1, three bacterial isolates (BSU-E6, BSU-E8, and BSU-E23) demonstrated the ability to produce IAA, solubilize phosphate, fix nitrogen, and produce ammonia. Therefore, these isolations were selected for further studies.

## Salt tolerance assay for the isolated endophytic bacteria

The ten endophytic isolates underwent an abiotic stress test in vitro at high salt concentrations (2.5%, 5%, 7.5%, and 10%). According to Table 2, the endophytic isolates displayed varying levels of salt tolerance, with growth decreasing at higher NaCl concentrations in the growth media. It was observed that three bacterial isolates (BSU-E6, BSU-E8, and BSU-E23) were able to tolerate high salt concentrations.

## Phylogenetic and identification of the selected endophytic bacteria

Three of the ten endophytic isolates were selected for further study based on their salt tolerance and plant growth-promoting characteristics. The 16S rRNA genes of these three isolates were amplified, sequenced, and compared to a database of known 16S rRNA sequences for molecular identification and phylogenetic analysis. The sequence analysis of approximately 1500 bp DNA fragments of the 16S rRNA genes was performed using nucleotide BLAST analysis. The first sequence (BSU-E6) showed a 99.72% similarity with *Streptomyces mutabilis*  (Figure 2) and was uploaded to GenBank with accession number PP496558. The second endophyte sequence (BSU-E8) had a 99.90% match with *Priestia megaterium* (Figure 3) and GenBank accession number PP496559. The third endophyte sequence (BSU-E23) exhibited a 100% similarity with *Bacillus pumilus* (Figure 4) and was assigned accession number PP496560. Additionally, phylogenetic trees for the 16S rRNA gene sequences were constructed using MEGA 11 software, employing the neighborjoining (NJ) method following sequence alignment with Clustal W (7.222) and default settings.

### Effect of bacterial inoculation on seed germination and seedling development under different salt concentration

Three endophytic strains-S. mutabilis BSU-E6, P. megaterium BSU-E8, and B. pumilus BSU-E23-were selected for an inoculation experiment with T. aestivum L. (wheat) seeds under salt stress conditions, both individually and in combination. The results demonstrated that these strains significantly improved seed germination percentages and seedling growth under salt stress. The germination percentage increased by 1.67% to 9.67% at a low NaCl level (100 mM), by 4% to 12.11% at a NaCl concentration of 200 mM, and by 5.11% to 16.78% at a concentration of 300 mM, compared to the uninoculated controls (Figure 5). After 40 days of inoculation with three selected endophytic bacteria, either individually or in combination (triple inoculum), as shown in Figure 6, the findings revealed the following: At 100 mM salt concentration, there was a significant increase (P < 0.05) in shoot length by 19-29%, shoot fresh weight by 4-9%, shoot dry weight by 6-8%, root length by 8-20%, root fresh weight by 8-12%, and root dry weight by 8-10%. At 200 mM salinity, the percentage of plants treated with PGPE strains that exhibited increased shoot length ranged from 13-35%, shoot fresh weight increased by 11-13%, and shoot dry weight increased by 9-11%. For root length, the increase was 10-28%, root fresh weight increased by 11-16%, and root dry weight increased by 12-16%. At 300 mM salt concentration, the increases were even more pronounced: shoot length increased by 32-55%, shoot fresh weight by 15-22%, shoot dry weight by 11-14%, root length by 14-37%, root fresh weight by 23-28%, and root dry weight by 13-18%, compared to control plants as shown in Table 3. These results suggest that *T. aestivum* seedlings may be effectively protected from salt stress-related damage by PGP endophytic bacteria.

Code	IAA production (µg/mL)	Phosphate solubilization Nitrogen fixation		Ammonia production	
BSU-E1	14.57 ± 0.58	-	-	+	
BSU-E3	-	-	+	-	
BSU-E6	37.79 ±0.51	+	+	+	
BSU-E7	12.78 ±0.68	-	+	+	
BSU-E8	58.57 ±0.50	+	+	+	
BSU-E13	-	-	-	-	
BSU-E16	8.68 ±0.54	-	+	+	
BSU-E18	16.36 ±0.49	-	+	+	
BSU-E22	-	-	-	+	
BSU-E23	56.42 ±0.83	+	+	+	

Table 1. Results of plant promoting activities of isolated bacteria. (+) indicates positive and, (-) indicates negative.

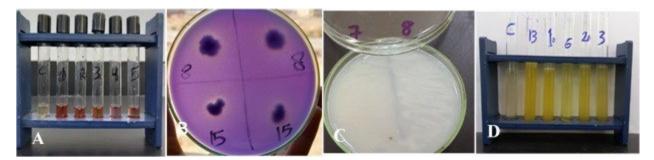
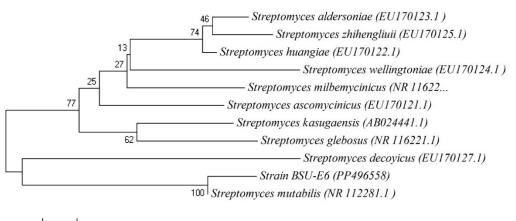


Figure 1. Plant Growth-Promoting Properties of Bacterial Strains A) Detection of IAA Production B) Detection of Phosphate Solubilization C) Detection of Nitrogen Fixation D) Detection of Ammonia Production.

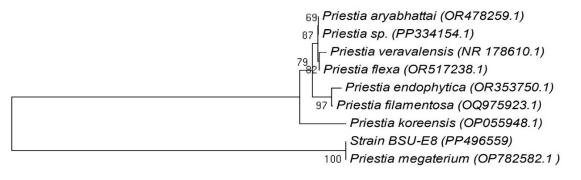
Table 2. Salt tolerance of bacterial endophytes isolated from Mentha longifolia, - refer to no growth and + normal growth.

Code	2.5 % NaCl	5 % NaCl	7.5 % NaCl	10 % NaCl
BSU-E1	+	+	-	-
BSU-E3	-	-	-	-
BSU-E6	+	+	+	+
BSU-E7	-	-	-	-
BSU-E8	+	+	+	+
BSU-E13	+	-	-	-
BSU-E16	-	-	-	-
BSU-E18	+	+	-	-
BSU-E22	-	-	-	-
BSU-23E	+	+	+	+



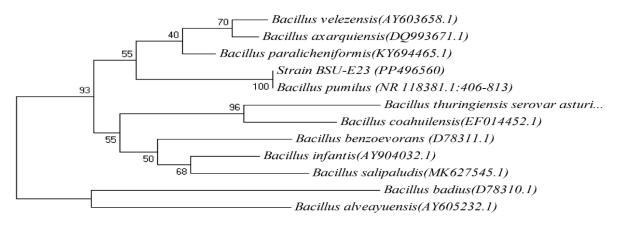
### 0.002

Figure 2. A phylogenetic tree was constructed for the bacterial isolated BSU-E6 using closely related 16S rRNA gene sequences acquired from NCBI. Numbers at the nodes represent bootstrap values from 1000 replicates, and the scale bar denotes 0.002% nucleotide divergence.



0.1

Figure 3. The phylogenetic tree for the bacterial isolate BSU-E8 was constructed using closely related 16S rRNA gene sequences obtained from NCBI. Numbers at the nodes represent bootstrap values from 1000 replicates, and the scale bar indicates 0.1% nucleotide divergence.



0.005

Figure 4. A phylogenetic tree was constructed for the bacterial isolate BSU-E23 using 16S rRNA gene sequences obtained from NCBI. Numbers at the nodes indicate bootstrap values from 1000 replicates, and the scale bar represents 0.005% nucleotide divergence.

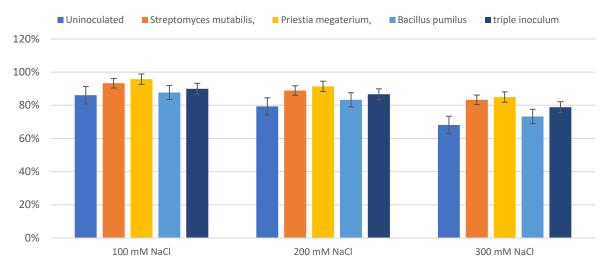


Figure 5. Evaluate the effects of three selected salt-tolerant endophytic bacteria, both individually and in combination (triple inoculum), on seed germination under varying salt concentrations



Figure 6. Effect of inoculation with selected endophytic bacteria and triple inoculum on seedling growth A) At 100 mM NaCl stress B) at 200 mM NaCl Stress C) at 300 mM NaCl stress C) uninoculated 1- S. mutabilis 2-P. megaterium 3- B. pumilus 4-Triple inoculum

Table 3. Effects of three selected salt-tolerant endophytic bacteria and triple inoculum on seedling growth under different salt cor	centrations.

Treatments	Root length	shoot length.	Fresh Weight (g)		Dry weight (g)			
	(Cm)	(Cm)	Root	Shoot	Root	Shoot		
100 mM NaCl								
Uninoculated	14.35±1.85	18.22±2.62	1.645±0.21	2.562±0.31	0.165±0.02	0.267±0.02		
Streptomyces mutabilis	15.52±1.62	22.83±2.11	1.778±0.19	2.745±0.21	0.178±0.01	0.286±0.01		
Priestia megaterium	16.86±1.78	23.12±2.16	1.832±0.17	2.776±0.23	0.181±0.01	0.287±0.01		
Bacillus pumilus	16.34±1.64	21.62±2.19	1.793±0.18	2.670±0.24	0.179±0.02	0.282±0.02		
triple inoculum	17.22±2.07	23.54±2.09	1.846±0.14	2.794±0.25	0.182±0.02	0.288±0.02		
200 mM NaCl								
Uninoculated	12.24±2.11	15.23±2.47	1.438±0.22	2.357±0.22	0.140±0.01	0.254±0.02		
Streptomyces mutabilis	14.79±1.96	18.95±2.60	1.611±0.14	2.645±0.18	0.159±0.01	0.280±0.02		
Priestia megaterium	15.31±2.09	20.18±2.66	1.647±0.17	2.652±0.20	0.161±0.01	0.281±0.01		
Bacillus pumilus	13.52±1.96	17.27±2.09	1.590±0.14	2.620±0.17	0.157±0.01	0.278±0.02		
triple inoculum	15.64±2.24	20.50±2.14	1.668±0.18	2.674±0.16	0.162±0.02	0.283±0.02		
300 mM NaCl								
Uninoculated	9.08±2.42	11.50±2.22	1.125±0.24	2.124±0.26	0.123±0.02	0.238±0.02		
Streptomyces mutabilis	10.33±2.09	15.21±1.91	1.396±0.15	2.448±0.29	0.141±0.02	0.265±0.02		
Priestia megaterium	11.76±2.43	16.56±2.11	1.415±0.16	2.467±0.31	0.142±0.01	0.269±0.01		
Bacillus pumilus	11.45±1.89	16.27±2.47	1.379±0.14	2.438±0.20	0.139±0.01	0.264±0.02		
triple inoculum	12.44±2.10	17.83±2.11	1.436±0.20	2.589±0.30	0.145±0.02	0.272±0.01		

### DISCUSSION

Endophytic bacteria may interact more closely with their hosts than rhizosphere and phyllosphere microbial populations. Due to their intimate relationship with plants, endophytic bacteria can directly and indirectly enhance plant health and growth, particularly in response to various biotic and abiotic challenges (Weyens et al., 2009). As the significance of these beneficial bacteria in improving plant quality becomes clearer, their potential applications for more sustainable agricultural production are being explored (Qin et al., 2014). Endophytic bacteria are found ubiquitously in various tissues of host plants, and some have been shown to positively affect plant health (Santoyo et al., 2016). In this study, we investigated the composition of endophytic bacteria associated with the roots and shoot tips of \*M. longifolia\* and reported the culturable bacterial endophytes isolated from these plants (Alaylar, 2022; El-Shatoury et al., 2006).

Ten bacterial isolates obtained from M. longifolia were evaluated for their plant growth-promoting (PGP) characteristics and salt tolerance. Among them, three isolates were selected: S. mutabilis, P. megaterium, and B. pumilus. Molecular identification of these isolates was performed using 16S rRNA partial gene sequencing analysis, a widely used method for bacterial taxonomy and phylogenetic studies (Jamali et al., 2020). According to Xu et al. (2016), the most prevalent genus in the endophytic actinobacterial communities of mangrove plants is Streptomyces. S. mutabilis is an endophyte with PGP and biocontrol gualities (Toumatia et al., 2016) and has also been shown to have antitubercular and antibacterial activity against bacterial infections (Yassien et al., 2015). P. megaterium, previously known as B. megaterium, has been discovered to withstand varying sodium chloride concentrations and produce plant auxin (Hwang et al., 2022). *B. pumilus* is categorized as a plant growth-promoting bacterium due to its properties and has been shown to stimulate plant development in salinity-stressed environments (De-Bashan et al., 2010; Kaushal et al., 2017; Kumar et al., 2021).

To identify bacteria with high potential for use as biofertilizers, the plant growth-promoting (PGP) properties of the bacteria were investigated. One major plant hormone is indole-3-acetic acid (IAA), and bacteria capable of producing IAA can enhance plant root growth and length, thereby creating a larger root surface area and allowing plants to absorb more nutrients from the soil (Islam et al., 2016). Rodrigues et al. (2016) demonstrated that, in the presence of 5 mM tryptophan, 57% of bacterial endophytes secreted high concentrations of IAA within 72 hours, ranging from 21.05 to 139.21 µg mL-1. Endophytic bacteria produced greater amounts of IAA compared to rhizosphere strains, indicating a potential symbiotic relationship and a closer bond between endophytes and their hosts (Taktek et al., 2017). Our findings are consistent with earlier studies that show inoculation with S. mutabilis enhances root and shoot growth, likely due to its growth-promoting compounds, such as IAA and phosphatase (Cruz et al., 2015). IAA synthesis and phosphate solubilization were demonstrated by B. pumilus TRS-3 (Chakraborty et al., 2013). Additionally, B. megaterium CDK25 was able to improve the nutritional and biological growth characteristics of *Capsicum* annuum L. by synthesizing 13.8 µg/ml (Bhatt & Maheshwari, 2020).

According to Bindraban et al. (2020), phosphorus is a limiting element in agriculture because plants cannot utilize it even though it is present in sufficient quantities in the soil. Endophytic microorganisms have been reported to be more successful at solubilizing phosphates compared to facultative and non-endophytic bacteria (Varga et al., 2020; Walia et al., 2017). In the current study, three bacterial isolates were found to be capable of dissolving inorganic forms of phosphate in qualitative phosphate solubility tests conducted on Pikovskaya's agar medium (Table 1). The formation of a clear area alongside the bacterial colonies may have resulted from the production of organic acids, polysaccharide synthesis, or enzymes responsible for phosphate solubility in these bacterial isolates (Paul & Sinha, 2015). According to Rodriguez et al. (2006), phosphate-solubilizing bacteria have been shown to enhance the uptake of phosphorus by plants, which

is a crucial aspect of plant nutrition. Several bacterial families, Bacillus, including Pseudomonas, Rhizobium, Agrobacterium, Acinetobacter, Enterococcus, Burkholderia, Acromobacter, Micrococcus, Aerobacter, Flavobacterium, Erwinia, Pantoea, and Enterobacter, possess phosphatesolubilizing capabilities (Anzuay et al., 2013). About 20% of the 300 actinobacterial isolates studied by Hamdali et al. (2008) were identified as members of the genus Streptomyces and were capable of solubilizing phosphorus. Several Streptomyces species isolated from the rhizospheres of tomatoes demonstrated the ability to produce high levels of phosphate (Anwar et al., 2016).

Here, most endophytic bacterial isolates were able to produce ammonia. According to Margues et al. (2010), bacteria that produce ammonia can provide nitrogen to the host plant. Furthermore, excessive ammonia production can act as a catalyst for the pathogenicity of opportunistic plant diseases. Numerous reports have documented the capacity of Bacillus species to fix atmospheric nitrogen (Saxena et al., 2020). Additionally, ammonia production has also been demonstrated in S. mutabilis (Passari et al., 2015). At higher concentrations of salt, three of the isolated endophytic bacteria exhibited salinity tolerance. Previous studies on endophytic microorganisms from desert-dwelling plants have also reported that bacterial endophytes possess high salt tolerance (Rashid et al., 2012). Plant growthpromoting endophytic bacteria (PGPEB) are found in various bacterial families, including Bacillus, Arthrobacter, Rhizobium, Burkholderia, Macrobacter, Klebsiella, Pseudomonas, and Oxytoca. These bacteria promote typical plant growth responses and mitigate the negative impacts of stressful growth conditions (Khan et al., 2020a). According to our findings, salt stress inhibits the growth and development of plants. However, the detrimental effects of salt stress can be significantly reduced by inoculating plants with selected endophytic bacteria. Their growth-promoting qualities enable these endophytic bacteria to positively influence wheat plants. Several researchers have highlighted the potential of PGPEB as a promising approach to alleviate salinity-induced plant stress, emphasizing the increasing importance of microorganisms in managing biotic and abiotic stress (Khan et al., 2019; Nadeem et al., 2010).

In conditions of saline stress, three strains were able to promote seed germination and seedling growth. Priming seeds with beneficial bacterial inoculums is a desirable ecological strategy to increase germination rates in the face of environmental stressors and to trigger early plant defense systems (Lastochkina et al., 2020). Priming generally impacts several physiological systems in seeds and plants and is characterized by primed plants' increased capacity to respond quickly and effectively to stressors (Singh et al., 2020). Understanding the mechanisms of action of Plant Growth-Promoting Bacteria (PGPB) is essential for developing effective procedures to mitigate the harmful effects of salinity on plants. Numerous interconnected direct and indirect pathways are responsible for the physiological effects of PGPB on plants (Lastochkina et al., 2019). mechanisms include These improving the bioavailability of macro- and micro-elements (e.g., nitrogen fixation, ammonia production, phosphate solubilization) to stimulate plant growth; inducing systemic resistance and tolerance to stresses; and producing phytohormones and regulating their levels in the host plant (Egamberdieva et al., 2017; Lastochkina et al., 2019b). Numerous studies have shown that beneficial bacteria can decrease the negative impacts of salinity on the growth and development of various plants, including wheat (Khan et al., 2020; Lee et al., 2021; Mahgoub et al., 2021; Shahid et al., 2022).

### CONCLUSIONS

The current study isolated endophytic bacteria from \**M.* longifolia\*, including \**S.* mutabilis\*, \**P.* megaterium\*, and \*B. pumilus\*. The selected bacterial endophytes exhibited salt tolerance and plant growth-promoting activities. These isolates can produce indole-3-acetic acid (IAA) and ammonia, fixing nitrogen, and solubilizing phosphorus. When wheat was inoculated with these selected strains, it promoted seed germination and seedling growth in saline environments. Therefore, bacterial endophytes can be used as bio-stimulators in sustainable agriculture, mitigating the effects of salinity stress and promoting the growth of various crop plants.

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