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Research Article

Plant growth-promoting endophytic bacteria isolated from *Launaea arborescens* (characterization and molecular identification)

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Abstract

The present investigation aimed to assess endophytic bacteria's capacity to stimulate plant development. Eight endophytic bacterial strains were isolated from the root of *Launaea arborescens* (Batt.), native medicinal plant growing spontaneously in the region of Bechar (south-west Algeria). The eight strains were tested for their effects on seed germination and plant growth of *Triticum aestivum* L**.** The results showed that all strains had positive effects on wheat seeds germination. The seeds inoculation of wheat by endophytic bacteria improves morphological parameters of the seedling (fresh and dry weight of plant, root and stem elongation). 16S rRNA gene sequencing was used to identify the powerful strain, it was found as *Brevibacillus brevis*. This one was found positive for many of the plant growth promoting attributes like ammonia production and azote fixing. In addition, it was identified as resistant to azithromycine during antibiotic sensitivity test and It could tolerate up to 0.9 M of NaCl. Additionally, *Brevibacillus brevis* was identified as resistant in the herbicide and the insecticide susceptibility test. Results suggest that this endophyte is a strong candidate for use as plant growth-promoting inoculants, to assist in lowering the amount of chemicals used in agricultural practices and enhance nutrient absorption and stress resistance in plant species.

Introduction

It is crucial to increase plant productivity because one of the main problems facing humanity is food insecurity on a worldwide scale. Synthetic fertilizers and chemical pesticides enable the production of crops with a high yield, but their widespread and continued use has raised concerns about upcoming environmental issues (Strassemeyer et al., 2017)**.** Therefore, establishing ecologically responsible and sustainable agriculture techniques is highly desirable. Agricultural chemicals, some of which have significantly improved the environment, can be replaced by microbial

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agents (Chen et al., 2020), by supplying crops with an abundance of nutrients and enhancing soil health and nutrient cycling (Karthik et al., 2016), and improving agricultural productivity as a result. Biologically, plant endophytes appear to be a genuine treasure trove. The most attractive possibilities are endophytic bacteria that support plant growth (PGP) and skills in biocontrol because they can colonize the inside of a plant and establish a reliable, long-lasting relationship with it (Rani et al., 2022). They create antibacterials and other compounds that can protect the plant from stressful situations. Without creating any disease signs, these microorganisms coexist in close proximity to the host plant (Pacifico et al., 2019; Azevedo et al., 2020). Additionally, endophyte bacteria are found in all plant tissues, and their potential to stimulate plant growth comes from either direct processes (like their capacity to fix nitrogen, produce ammonia, produce plant hormones, and dissolve phosphate and potassium) or indirect impacts (secretion of extracellular enzyme, siderophores and 1-aminocyclopropane-1-carboxylate deaminase) (Hassan et al., 2017). However, endophytes from medicinal plants have garnered interest because of their wide range of bioactive metabolites. (Toghueo et al., 2017). *Launaea arborescens* (Batt.) (Asteraceae) is widely used in traditional medicine. The latex from its stems is applied with a brush to treat dermatological conditions and hemorrhoids. On treat a child's fever, the crushed stems are applied as a poultice to the head and in order to treat stomach problems, stem powder is used. Despite *Launaea arborescens*' usefulness in medicine and the related bacteria's potential for bioactivity, no studies on its endophytic bacteria have been conducted to date. This study sought to collect several endophytic bacteria from *Launaea arborescens* in order to assess their capacity to promote plant development and their potential role in stress management.

Materials and methods

Sampling of plant and bacterial endophyte isolation

Endophytic bacteria were isolated from fresh roots of healthy wild medicinal plant (*Launaea arboresens)* collected from the region of Bechar (south-west Algeria). Plant roots were collected, packaged sterilely, and brought to the lab where they were treated right away. In order to get rid of any debris, plant roots were rinsed under flowing water for 3–5 min and then surface sterilized for 1 min with 70% ethanol, then washed for 4min with 3.8% sodium hypochlorite, followed by 30 sec of 70% ethanol, and finally rinsed three times with sterile distilled water to remove hypochlorite (Dalal & Kulkarni 2013). Using a sterile mortar pestle, the samples that had been surface-sterilized were macerated for 20 to 30 min in sodium chloride (0.9% NaCl). In nutrient-agar plates, the macerate dilution (10^{-1} and 10^{-2}) was applied and incubated for 3 to 7 days at 28°C (Hazarika et al., 2021). Based on colony morphology, bacteria were isolated and streaked over nutrient agar plates until a certain purity level was reached. The following formula was used to determine the microbial population (Alyexandra, 2020):

$$N\ (UFC / g) = \sum C/V[n1 + (0{,}1x\ n2)]\ d$$

Where:
N = colony counts per gram of sample (units = $cfu.g^{-1}$).
$\sum C$ = total of all the colonies in all of the plates.
V = volume of inoculum applied to each disk (in ml).
n1= number of dishes retained at first dilution.
n2= number of dishes retained at second dilution.
d = dilution factor corresponding to the first dilution retained.

Bacterial strains' impact on the germination of seeds

Eight endophytic bacterial strains were found, and their capacity to promote the germination of wheat seeds (*Triticum aestivum* L) was evaluated in vitro in Petri dishes by using the method of Aiter (2015) (slightly modified). Wheat seeds were surface disinfected for 10 minutes with 6% sodium hypochlorite, followed by three sterile distilled water rinses. The surface disinfected seeds were immersed for 1 hour in 20 ml of each overnight bacterial

culture (adjusted to 10^8 CFU/ml using UV-visible spectrophotometer at OD_{600}). After drying on sterile blotting paper, the seeds were sown in Petri dishes containing water agar (7 g/ L). The operation was repeated for five seeds. Untreated seeds served as a control. The Petri dishes were put for 5 days in a 24 °C climate-controlled environment and 60–70% relative humidity with 16 h light (3000 lux) and 8 hours dark conditions. The following formula was used to get the germination percentage (Kerbab et al., 2021):

Germination rate (%) = (number of seeds that grew / total number of seeds inoculated) × 100

Bacterial strains' effects on the stimulation of plant growth

All the dishes used to evaluate the effect of the isolates on the improvement of seed germination were recultivated until the 10th day in order to study the effect of the isolates on the promotion of plant growth. After cultivation, the vitro-seedlings were carefully uprooted and the roots were washed and freed from adherent agar in order to measure the growth parameters. Five replicates of each treatment were collected and growth indicators including root length (RL), stem length (SL), plant fresh mass (PFW), plant dry mass (PDW) (only PDW which has been recorded after they had been drying the seeds in an oven at 105°C till the weight remained constant) were recorded for each wheat seedling and compared with those of the control (Aiter, 2015).

Characterization of potent isolate
Molecular identification

Potential isolate was identified using 16S rRNA gene. The GF-1 Nucleic Acid Extraction Kit (Vivantis Technologies Sdn Bhd, Selangor DE, Malaysia) was used to extract the genomic DNA of bacteria according to the manufacturer's instructions (González-Rodríguez et al., 2016).

The universal primers 16F27 (forward) (5'-AGAGTTTGATCCTGGCTCAG-3') and 16R1492 (reverse) (5'-CCGTCAATTCCTTTGAGTTT-3') were used to amplify the 16S rDNA (Edwards et al., 1989). 50 µl of master mix were used in the PCR reaction mixture (1.25 U Hot Start Taq DNA Polymerase (Solis Biodyne, Estonia), 25–50 ng/µl µl of DNA template, 0.3 µM µl of each primer, 1.5 µM $MgCl_2$ Magnesium chloride (Solis Biodyne, Estonia), and made up to 50 µl reaction volume with distilled H_20. The following conditions were used for PCR: Initial denaturation (at 94 °C for 12 min); Denaturation (at 94°C for 30 s); Annealing (at 55°C for 30 s); Extension (at 72°C for 1 min 40 s). 30 cycles of amplification were performed. Subsequently, a last extension was made at 72°C (7 minutes). PCR was conducted using a thermocycler (iCycler Bio-Rad, USA). DNA concentrations were checked using Nanodrop Spectrophotometer (NanoDropTM 2000, USA).

Analysis of the amplified DNA was done by horizontal gel electrophoresis into a 1.5% agarose gel (Sigma-Aldrich, USA). As DNA molecular weight markers one hundred base pair (100 bp) DNA ladder (Solis Biodyne, Estonia) was used. Electrophoresis was done for 1 h 30 min at 80 V, and after being stained with Midori Green Advance, the gel was examined under UV lighting. (Nippon Genetics, Japan) and inspected with a UV transilluminator. The Clean-Up kit (Vivantis) was used to purify the PCR products.

Sequencing of the purified PCR products was performed in the forward and reverse directions in separate reactions and duplicates. For each reaction, the following ingredients were used: 2 µl of the appropriate PCR primer, 40 µg template DNA, 10 µl water, and 2 µl BigDye Terminator v3.1 Ready Reaction Mix (Applied Biosystems). Each reaction was heated for 1 min at 96°C, followed by 25 cycles (96°C for 10 s, 50°C for 5 s, and 60°C for 4 s). With an ethanol precipitation method, the sequencing products were purified to get rid of any unincorporated reagents and ensure a neutral charge. Briefly, 80 µl of an ethanol precipitation mix was used to wash the sequencing products (62.5 µl 95% ethanol, 3 µl NaAc, and 14.5 µl water) and by centrifugation (15 min/13000 rpm) the DNA was pelleted. The pellet was again washed in 200 µl 75% ethanol and centrifuged (15 min/13000 rpm). A 3130 Genetic Analyzer Capillary Array (Applied Biosystems) was used to load the pelleted DNA for detection after it had been air-dried and rehydrated in 15 µl formamide. Using Bionumerics v3.5 (Applied Maths), two forward and two

reverse sequences for each sample were aligned to obtain a composite sequence. Each sequence trace's quality was evaluated visually, and the low-quality sequences were modified and eliminated. Using BLASTn (available on the NCBI website), Consensus sequences were compared to a database to identify organisms (http://blast.ncbi.nlm.nih.gov) (Kumar et al., 2015).

Morphological and biochemical characterization

In this part, morphological characteristics (Gram stain, motility and colony color) as well as biochemical characterization (catalase, oxidase, nitrogen fixation, ammonia production and phosphate solubilization activities) of the potent isolate were carried out.

The molecular nitrogen-fixing activity was examined in a solid nitrogen-free media. The bacterial cultures were streaked onto the Wickerham salt medium, which was then incubated for 48 hours at 30° C. Any development on this surface demonstrates the capacity of microorganisms to fix nitrogen (Bouras, 2018).

The peptone water was tested for ammonia production. 100 µl of freshly developed culture were injected in 10 ml of peptone water and incubated at 30 ± 2°C for 48–72 hours. As a control, peptone water without bacterial inoculation was utilized. Nessler's reagent was added in the tube along with 0.5 ml. A brown to yellow hue change was a sign that ammonia was being produced (Singh et al., 2014).

The ability of bacteria to solubilize phosphate was evaluated by inoculation with spots on the Pikovskaya media (containing tricalcium phosphate) and incubated at 28 ± 2 °C for 2–3 days. Clear halo zones that developed around the strains demonstrated their effective phosphate solubilization activity (Shahid et al., 2015).

Abiotic stress tolerance

The evaluation of antibiotic sensitivity of potent isolate

The disc diffusion method was utilized for antibiotic sensitivity testing to identify the patterns of sensitivity and resistance of a selected bacterial strain. Four drugs, including streptomycine (300 µg), spiramycine (100 µg), azithromycine (15 µg), and ciprofloxacine (5 µg), were used.

In order to conduct this test, a Mueller-Hinton agar plate surface was inoculated with bacteria (10^8 CFU/ml). On the surface of the inoculated agar, the antibiotic disks were applied. The zones of growth inhibition surrounding each antibiotic disk were measured to the nearest millimeter after incubation period (24 h at 30°C), and they were then contrasted with the range specified by Bauer et al. (1966).
Where: susceptible: >15 mm; intermediate: 10–15 mm; resistant: <10 mm.

Salinity tolerance of potent isolate

The selected bacterial strain was tested for its salt tolerance ability by inoculated by streaking on nutrient-agar containing different concentrations of NaCl (ranging from 100, 300, 500, 700 and 900 mM), incubated at 30°C for 24 hours and observed for growth. Growth on these media reflects the ability of bacteria to tolerate different concentrations of NaCl (Rojas-Tapias et al., 2012).

Herbicide and insecticide tolerance test

The method of McKeen, Reilly and Pusey (1986) was slightly modified to evaluate the selected bacterial strain for its tolerance to herbicide and pesticide. In this procedure, agar plates were inoculated with a standardized inoculum (10^8 CFU/ml) of the endophytic bacteria. Then, sterile filter paper discs (about 6 mm in diameter) were soaked in the dilute solution of insecticides (1/200 of Majic: Alpha-cypermethrin at 10% (w/v)) or herbicides (1/100 of Tiller: Glyphosate acid at 410 $g.L^{-1}$). Discs were drying at laboratory temperature and placed on the agar surface. After incubation at 30°C for 24 hours, sensitivity to the insecticide or herbicides was manifested by the appearance of zones of inhibition.

Results

Isolation of endophytic bacteria

In our study, endophytic bacteria were successfully isolated from roots of the *Launaea arborescens* using spread plate method. Nutrient agar plates inoculated with *L. arborescens* root

samples showed morphologically different colonies. Population of endophytic bacteria associated with the root of this plant was $13.6.10^3$ cfu.g^{-1} fresh weight. Therefore, eight endophytic bacterial strains, coded: Lar01 to Lar08, were isolated based on the morphological characteristics of the colonies on the plates (of which Lar05 was orange-red, Lar06 was orange and the others were white).

Bacterial strains' impact on the germination of seeds

The effect of bacterization treatment of wheat seeds on the germination rate is summarized in Table 1. The results indicated that all strains influenced the germination of wheat seeds favorably. In effect, the germination rate is variably affected by the treatment of the grains by the different isolates. A maximum germination rate (100%) was obtained by strain Lar01, Lar03, Lar04, Lar05 and Lar08. Isolates Lar06 and Lar07 clearly improved the germination power of seeds like that of the control with a germination rate of 80%. The Lar02 strain appears in this case to be less efficient than all the isolates, causing a low germination rate of 60%.

Table 1. Seed germination and growth promotion effects of bacterial strains

	Parameters				
Selected strains	Germination rate (%)	Stem length (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Lar01	100	10.65	7	0.83	0.03
Lar02	60	10.5	10	0.98	0.12
Lar03	100	11.10	10	0.85	0.08
Lar04	100	13.5	11.30	0.91	0.13
Lar05	100	12.68	12	0.91	0.13
Lar06	80	14	11	0.46	0.05
Lar07	80	11.12	11	0.81	0.13
Lar08	100	14	10.82	0.81	0.10
Control	80	9.25	9.25	0.79	0.08

Bacterial strains' effects on the stimulation of plant growth

For the effect of bacterial strains on final average length the stem and the root and according the results obtained (Table 1), we noticed that the bacterization of the wheat seeds caused a clear difference in the length of the stem and the root of the seedling compared to the unbacterized controls. The best length of the rod was recorded under the effect of strains Lar06 and Lar08 and the values are between 10.5 to 14cm compared to the control (9.25cm) (Figure 1). Similarly, the best length of the root was recorded under the effect of strain Lar05 and the values are between 10 to 12 cm compared to the control (9.25 cm). Strain Lar01 was found to be ineffective on root length.

On the impact of bacterial strains on the average fresh and dry weight of the seedlings, the results obtained indicated that for the majority of the strains tested, the bacterization of wheat seeds induces a clear difference in the fresh and dry mass of the seedlings treated in comparison to the controls. Fresh seedling biomass was improved by all strains except Lar06. The values are from 0.81 g to 0.98 g in comparison to the control (0.79 g). Likewise, the dry biomass of seedlings was improved by all strains except Lar01 and Lar06. The values were from 0.08 g to 0.13 g compared to the control (0.08 g). These findings reveal that among the isolates tested in this primary screening, the strain Lar08 has proven the best effects.

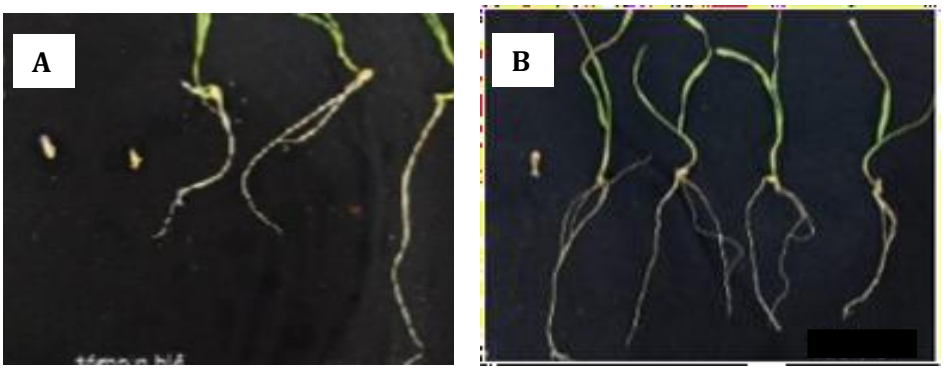

Figure 1. Effect of Lar08 stain on promote seedling emergence and growth of Triticum aestivum A: Control ; B: Triticum aestivum + Lar08

Characterization of potent isolate

Molecular identification

Both strands of the DNA of the powerful isolate (Lar08) were sequenced for molecular identification. The sequence is accessible under the Genebank accession number OP994028 after being submitted to the National Center for Biotechnology Information (NCBI). (https://www.ncbi.nlm.nih.gov/nuccore/OP994028). The length of the amplified gene sequence was 1377 bp. When the bacterial isolate's sequenced PCR results were compared to the 16S rRNA sequences present in the GenBank database, they revealed a 99.77% similarity to Brevibacillus brevis. The phylogenetic tree constructed using a bootstrap Neighbour-joining method based on 16S rRNA gene sequencing is depicted in Figure 2.

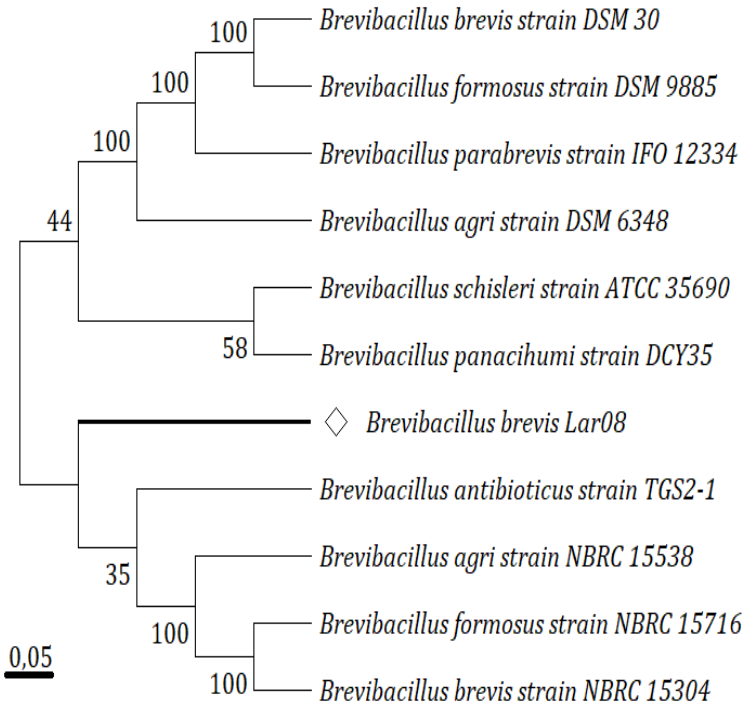

Figure 2. Phylogenetic analysis of bacterial strains' 16S rRNA sequences using NCBI reference sequences. MEGA 11 was used to do the analysis, and the neighbour-joining approach was employed.

Morphological and biochemical characterization

The isolate Lar08 strain was gram positive, motile rod. It was punctiform with a raised elevation and an entire margin. The colony was creamy white in color with a rough surface. It has shown positive results for the nitrogen fixation test, ammonia production test and catalase test. It produced negative results for phosphate solubilization, oxidase test, and could not ferment mannitol (Table 2).

Table 2. Biochemical and morphological characterization of Lar08 strain

Test	Lar08 strain	Test	Lar08 strain
Gram staining	Positive	Catalase	Positive
Colony color	Creamish white	Mannitol	Negative
Shape	Rod	Nitrogen fixation	Positive
Mobility	Positive	Ammonia production	Positive
Oxidase	Negative	Phosphate solubilization	Negative

Abiotic stress tolerance of potent isolate

The evaluation of antibiotic sensitivity of potent isolate

The sensitivity of potent isolate against different antibiotics in terms of the zone of inhibition is presented in Table 3. The results showed that the strain was identified as resistant to Azithromycine and sensitive to Streptomycine, Spiramycine and Ciprofloxacine.

Salinity tolerance of bacterial strains

The selected bacterial strain was tested for its tolerance to salt stress by exposing the bacterial cells to various NaCl concentrations. The detailed responses are presented in Table 3. The results showed that the strain had grown at the concentration of 0.5, 0.7 and 0.9 M of NaCl.

Herbicide and insecticide tolerance test

The results obtained showed resistance to the herbicide for the strain tested translated by the absence of a zone of inhibition around the discs of the herbicide, which means that there is a tolerance to Tiller (410g.L^{-1}) for this bacterium (Table 3). Here, it was observed that the insecticide Majic at 10% concentrations was easily tolerable for the strain tested (Table 3).

Table 3. Abiotic stress tolerance of endophytic potent isolate (Lar08)

Strain	Antibiotics inhibition zone (mm)				NaCl concentrations (M)					Insecticides inhibition zone (mm)	Herbicides Inhibition zone (mm)
	Azir	Cip	Sp	Str	0.1	0.3	0.5	0.7	0.9	Majic (10%)	Tiller (410g.L^{-1})
Lar08	00 (R)	24 (S)	22 (S)	19 (S)	-	-	+	+	+	00 (R)	00 (R)

Azir : Azithromycine (15 µg/disc) ; Cip : Cipromycine (5 µg/disc); Sp : Spiramycine (100 µg/disc) ; Str : Streptomycine (300 µg/disc) ; S : sensitive; R : Resistance ; + : bacterial growth; - : no bacterial growth.

Discussion

In comparison to other epiphytic bacteria, endophytic strains have superior colonization efficiency, which makes them an important resource for disease and growth management in plant systems. In sustainable agriculture, endophytic bacteria are emerging as valuable substitutes for biochemical pesticides since they are a source of natural bioactive chemicals. This study's main objective was to isolate bacterial strains from the roots of the medicinal plant *Launaea arborescens*, and to assess whether those strains improve the germination of seed and seedling development as well as

their possible approach in stress management. It should be mentioned that numerous researches have documented the isolation of endophytes from the Asteraceae family. (Zhang et al., 2019; Amr et al., 2021). However, to the authors' knowledge, no previous studies focused on endophytic populations in *Launaea arborescens* plant.

The bacterial population obtained was observed to be within the range of endophytes previously reported in many previous studies (Elvira-Recuenco & Van Vuurde, 2000). Dalal and Kulkarni (2013) assert that the species, age, tillage, and environmental factors have an impact on the population of endophytic bacteria. Compared to previous growth stages, the microbial population is at its highest during the reproductive period. Different maturity phases affect the diversity of microbial endophytes, which in turn affects the kind and quantity of root exudates. To be more precise, the host plant species' characteristics have a significant impact on the type of endophytic community that a plant has (Ding & Melcher, 2016). In fact, the endophytic variety of various plant species growing in the same soil might vary significantly (Germida et al., 1998). Additionally, the technique utilized to investigate these bacteria, such as the type, concentration, and even the length of the treatment period for a sterilizing agent used to recover bacteria, affects the endophytic diversity of plants.

The heavy use of agrochemicals and management with a high environmental effect, that compromise soil fertility and crop development, have long been hallmarks of *Triticum aestivum* L. production techniques. In the current study, seeds of *T. aestivum* were treated with endophytic bacteria to verify their ability to promote seed germination. The results showed that all strains had positive effects on the germination of wheat seeds. These findings concur with those of numerous researches, including those on *Brevibacillus brevis* (Nehra et al., 2016; Sondang et al., 2021).

Endophytic bacteria were also evaluated for their ability to promote seedling emergence and growth. It is observed that a few days after the treatment, a significant effect of the microorganism inoculum was observed in the seedlings, with an increase in the size, the fresh and dry biomass of the seedlings compared to the untreated wheat seedlings. These findings concur with those of numerous researches, including those on *Brevibacillus brevis* (Nehra et al., 2016; Sondang et al., 2021). Endophytic bacteria are assumed to be responsible for the increase in plant growth that results from their ability to boost nitrogen fixation, photosynthetic activity, and indole acetic acid synthesis (Nongkhlaw & Joshi, 2014). Other beneficial effects of growth are connected to endophytic, including aiding in osmotic management, stomata, morphological adjustment, greater mineral uptake, and modifications in nitrogen buildup and metabolism. So, to increase plant development and yields, isolates demonstrating PGP activities can be utilized as soil or plant inoculants. *Brevibacillus brevis* is one endophytic microbial strain that has been utilized regularly over the past 20 years in order to stimulate plant development and control biotic and abiotic stress (Kumar et al., 2014; Kumar et al., 2016; Nehra et al., 2016).

Analysis of the 16S rRNA gene fragment of strain Lar08 showed its 99.77% similarity to *Brevibacillus brevis* (Firmicutes). Several studies have demonstrated that *Brevibacillus brevis* is associated as endophytic bacteria with medicinal plants: *Pulicaria incisa* (Amr et al., 2021), and crops plants: cotton (Nehra et al., 2016) and rice (Sondang et al., 2021). The presence of *Brevibacillus brevis* as endophyte bacteria in crop plants allows us to say that this species is not pathogenic for humans. In addition, previous studies have shown the ability of *Brevibacillus brevis* to grow as an endophyte after inoculation into crop plants as a new host (Soad et al., 2005).

In our study, potent strain had the ability to produce ammonia and it was able to show nitrogen-fixing activity. Nitrogen fixation is a crucial characteristic that can directly affect plant growth. Endophytic bacteria can increase the nitrogen supply for their host plants. By exhibiting nitrogenase activity, these microbium is capable of giving their host plants fixed atmospheric nitrogen. All N2 fixing bacteria possess the highly conserved protein nitrogenase, and there is strong evidence to support lateral gene

transfer (Ivleva et al., 2016). Under controlled circumstances, nitrogen-fixing bacteria have been shown to boost the biomass of the host plant by N2. Nitrogen (N_2) from the atmosphere is biologically fixed into assimilable forms by diazotrophic bacteria, who convert it to ammonia via nitrogenase. Plants utilize ammonia as a source of nitrogen for growth. These findings are consistent with the majority of research on the characteristics of PGPB. *Brevibacillus brevis* showed very good ammonia generation capability (Nehra et al., 2016). Additionally, the selection of bacteria that support plant growth is frequently based on the synthesis of ammonia by microorganisms (Srivastava et al., 2014).

The potent isolate was further tested for its antibiotic susceptibility against four different antibiotics. Antibiotic-impregnated discs (6 mm diameter) were utilized for this test. The Kirby Bauer disc-diffusion method was used to assess the strains' antibiotic sensitivity to azithromycin, spiramycin, cipromycin, and streptomycin. According to Bauer et al. (1966), organisms were classified as resistant, intermediate, or sensitive based on the inhibition zones. In effect, the findings of our investigation showed a differential response of bacterial strain towards antibiotics. The strain was resistance to azithromycine and no resistance was observed against cipromycine, spiramycine and streptomycine. As in clinical impact strains, genes for antibiotic resistance can also be identified in environmental bacteria and their transfer occurs from one bacterium to another horizontally (Larsson & Flach, 2022). One of the primary causes of antibiotic sensitivity in this situation may be the fact that endophytic bacteria are less exposed to humans than they are to inner plants (Liotti et al., 2018; Afzal et al., 2019).

In the present research, the selected strain grew at high NaCl concentrations up to 0.9 Mol.l^{-1}, and it may be regarded as a facultative haplotype for resistance to abiotic stressors, particularly salinity and drought. Furthermore, the capacity of bacteria to withstand high NaCl concentrations may be a crucial characteristic for displaying their PGP activities in plants cultivated in saline environments. Compared to other studies and according to Amr et al. (2021), *Brevibacillus brevis* had great salt tolerance up to 200 mM, and increasing the NaCl concentration over this point reduced their development. In contrast, compared to bacteria isolated from non-saline habitats, those from arid or salty environments can endure inhibitory salt concentrations (Tripathi et al., 2018).

The selected strain was tested for its tolerance to the herbicide and insecticide namely Majic and Tiller respectively. Pesticide-impregnated discs (6 mm diameter) were used in this test. In our experiment, Majic herbicide was tolerated by the strain tested. There may be adaptation mechanisms that don't require particular selected agents to act as inducers, according to Rovida et al. (2021). On bacterial strains possessing tolerance systems without preceding selection processes, no papers could be located. In communities arranged as biofilms, sensitive bacteria can be efficiently shielded, where a portion of the population may have tolerance mechanisms but are chosen by stressors, as in the case of marine periphytic biofilms subjected to the ecotoxicological effects of copper (Corcoll et al., 2019).

During insecticide tolerance test and based on the inhibition zones, the selected strain had resistance to Tiller. It should be noted that some that some pesticides may not affect the growth and survival of these bacteria, even at very high dosages. On the other hand, some pesticides can poison helpful bacteria even at very low levels (Shahid & SaghirKhan, 2022). Therefore, having a higher tolerance threshold is important for a number of reasons, including the fact that pesticide-tolerant isolates can flourish in an environment where there is pesticide stress and that, if used as inoculants while there is pesticide stress, they can improve the growth performance of cultivated plants (Shahid et al., 2021). In addition, the fact that many toxins and pesticides can be metabolized and degraded by plant-associated microbes raises serious concerns regarding their usage in biotechnology.

Globally, *Brevibacillus brevis*, a PGPR that is widely distributed in soil and sediment and has several potential uses, has been heavily used in environmental remediation and agriculture throughout the world. Numerous researchers

have employed this bacterium as a PGPR on various crops (Nehra et al., 2016). *Brevibacillus brevis* has a high secretion capacity for secondary metabolites, which are crucial for pathogen control. Among these, tyrocidine, edeine, gratisin, and gramicidin have all been consecutively isolated and identified (Che et al., 2013). Because of its antagonistic activities against pathogenic bacteria and fungus, *B. brevis* may be an excellent choice for use as an antibacterial agent to improve crop yield and quality.

Conclusion

The results from the present study point out that *Launaea arborescens* is associated with potential endophytic bacteria (*Brevibacillus brevis*), this may be used in the future as a potential alternative for compounds that promote plant growth. A *Launaea arborescens*-associated endophytic isolate was discovered to fix nitrogen, create ammonia, and have favorable effects on wheat seed germination and increased seedling growth. These traits are added to its tolerance to abiotic stress. This isolate's content allows for the creation of novel inocula for use in agriculture and industry.

Author's declaration and contribution

The authors show no conflict of interest and equal contributions have been made by each author.

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