
Potential effect of root-associated bacteria from *Origanum vulgare* L. on wheat and tomato seedlings

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Abstract Medicinal plant root bacteria present multifunctionality and can act as important stimulator agents for their host plant. Eight strains recovered from the roots of *Origanum vulgare* L. medicinal plants growing in Ben Djerrah, Guelma, Algeria, were classified into the genera *Bacillus*, *Burkholderia*, *Rhodococcus*, and *Pseudomonas*. The functional traits for amylase, lipase, and indole-3-acetic acid (IAA) production were detected in all strains, while 75% were positive for hydrolytic proteases. Particular emphasis is placed on strains EpB1 and EdB8, which were efficient enzyme producers and exhibited the highest IAA production (114.56 ± 0.27 µg/ml and 114.06 ± 0.44 µg/ml, respectively). There was a significant enhancement in the growth of wheat and tomato seeds pretreated with the isolates *Bacillus cereus* EpB1, *Pseudomonas luteola* EdB6, and *Bacillus subtilis* EdB8. They enhanced the seed germination rate, vigor index, and the shoot and root lengths and biomass of seedlings in comparison to the control. Accordingly, the epiphytic strain EpB1 and the endophytic strains EdB8 and EdB6 from *Origanum vulgare* L. roots could potentially be used as biofertilizers to manage wheat and tomato cultivation.

Keywords: Endophytic and epiphytic bacteria, Enzyme activity, Indole acetic acid, Medicinal plant, Plant growth promotion

Introduction

A large microbial population colonizes the roots of all plant species. These microorganisms can be present both as epiphytes on surfaces and as endophytes in tissues without inducing any apparent effects (Passari *et al.*, 2015). According to various studies, plants benefit from the bacteria that form a symbiotic interaction with their roots (Abbamondi *et al.*, 2016; Silva *et al.*, 2020). Plant growth-promoting bacteria (PGPB) are defined as a living bacterial community around the rhizosphere that controls and enhances plant growth through several processes (e.g., biofertilization and biocontrol)

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(Herrera-Quiterio *et al.*, 2020). The auxin production is one of the direct mechanisms that can improve various plant development stages, such as root system formation and architecture modulation (Herlina *et al.*, 2017). The auxin indole-3-acetic acid (IAA) is the main naturally occurring and well-studied form. Bacteria have multiple mechanisms for synthesizing IAA, the tryptophan serves as the base compound, including IAA biosynthesis via indole-3-pyruvate (IPyA), which is the pathway used by bacteria to stimulate plant growth (Ahmed and Hasnain, 2014). Other beneficial compounds like siderophores and lytic enzymes constitute indirect processes that can have several regulatory effects on plant growth (Joe *et al.*, 2016). These properties meet the requirements of sustainable agriculture, making bacteria with these advantages a better alternative that protects both the plant and its environment (Silva *et al.*, 2020).

Medicinal plants have many bioactive molecules produced by their microbiota. Therefore, they may be reservoirs of pertinent biotechnologically bacterial isolates (Passari *et al.*, 2015). One of the most important medicinal aromatic plants in Algeria is *Origanum vulgare* L. (*O. vulgare*), which is a highly polymorphic species in the *Lamiaceae* family. This species often grows in the Mediterranean area. It represents one of the most important essential oil-producing species. In Algeria, people used it as a culinary herb, and it is also used as an antimicrobial, insecticidal, antiherbivore, and antioxidant agent (Mastro *et al.*, 2017). The use of root microbiome from medicinal plants is very crucial, as they provide beneficial effects on plant development, productivity, and the production of metabolites of industrial importance, as well as an essential key in controlling plant pathogens (Benaissa *et al.*, 2018). Given the characteristics of *O. vulgare*, we suggest that the bacteria related to the roots of this plant may positively contribute to agriculture.

Wheat (*Triticum durum*) and tomato (*Lycopersicum esculentum*) are essential crops that are cultivated worldwide. In Algeria, they are widely consumed and used as raw materials in the manufacturing of a variety of products. During recent decades, their production and yield have been severely affected by a diverse range of factors (disease, use of chemicals, climate change, etc.). Consequently, the advantage of inoculating their seeds with PGPB is that they will act as an effective and ecological tool for protecting the plant from pathogens and other environmental stresses and establish populations that will have a competitive advantage over organisms invading the rhizosphere (Choudhary *et al.*, 2021; Samaras *et al.*, 2021). Recently, Castronovo *et al.* (2020) characterized the cultivable isolates found connected with diverse parts of the *O. vulgare* L. and demonstrated their antimicrobial activities against pathogens. However, for crop seedlings, data on the functional

traits linked to increased crop production by this community are limited. Therefore, in this present research, some lytic enzyme- and IAA-producing bacteria isolated from the roots of *O. vulgare* L. were evaluated for their influence on the germination and seedling of wheat and tomato seeds.

Materials and methods

Study region and sample collection

The radicular parts of four *Origanum vulgare* L. individuals were sampled in the Ben Djerrah region ($36^{\circ} 25' 56''$ N, $7^{\circ} 22' 7''$ E), situated in Guelma Province, northeastern Algeria (Figure 1). Nestled in the heart of the Babors mountain range, the area is characterized by a Mediterranean climate, hot in summer and cold in winter. The mean annual temperature ranges from 4°C to 35.4°C , with an average of 17.3°C . The roots were collected on the same day, February 20, 2024. Roots of uniform size free from visible symptoms of disease or external destruction were collected. Overly mature or too young roots were excluded (Sánchez-López *et al.*, 2018). The samples were transported under aseptic conditions to the Microbiology Laboratory of Guelma University for analysis.

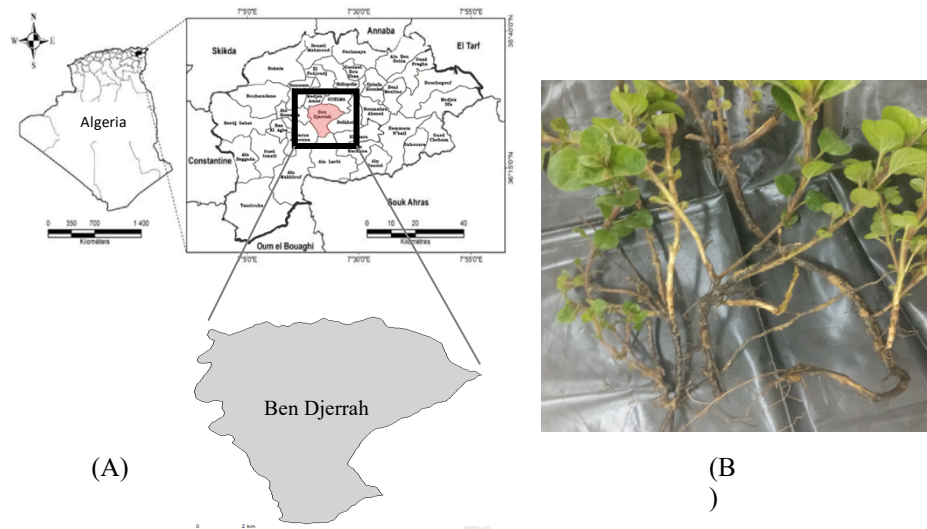


Figure 1. Area of sample collection (A) of *Origanum vulgare* L. roots (B)

Phenotypic characterization of epiphytic and endophytic bacteria

After removing all clods and soil from the root fragments by water washing, epiphytic bacteria (EpB) were extracted by shaking for 1 minute in sterile distilled water. Aliquots of 0.1 ml were streaked out on three culture media: Luria Bertani agar (LBA), King's B (King B), and Nutrient agar (NA) (Merck, Germany) (War Nongkhlaw and Joshi, 2014). For endophytic bacteria (EdB), root surfaces were disinfected in two steps with 70% ethanol (30 seconds each), separated by a 3-minute treatment with 5% sodium hypochlorite. The roots were also soaked in a 10% sodium bicarbonate solution for 15 minutes to disrupt and inhibit the growth of endophytic fungi. The roots, washed four times with distilled water, were then ground in 90 ml of sterile physiological saline (180 rpm for one hour at ambient temperature). Sub-volumes of 50 µl were plated on the surfaces of the three previously mentioned culture media and incubated at 30 °C for 48 hours. Additionally, a 100 ml aliquot from the last flush was inoculated into a nutrient broth at 28 °C for 24 hours to assess the efficacy of the disinfection process. Colonies exhibiting different macroscopic appearances are subcultured on the same isolation medium and phenotypically identified (Kurabachew and Wydra, 2013).

Enzymatic characterization of bacteria

The strain's ability to produce extracellular enzymes was investigated. Amylase, protease, esterase, lipase, urease, and gelatinase were detected by inoculating the bacterial strains in NA medium containing 0.2% soluble starch, YEPD medium (yeast extract peptone dextrose) supplemented with 2% casein (Silva *et al.*, 2020), NA supplemented with cholesteryl oleate and calcium chloride (Herrera-Quiterio *et al.*, 2020), tween 80 medium (Kumar *et al.*, 2012), urea broth (Zhao *et al.*, 2019), and mineral salt agar medium containing 1% gelatin (Merck, Germany), respectively (Fouda *et al.*, 2015). Most enzyme activities appear after 48-72 hours of incubation at 28-30°C as a translucent zone around the colonies. Only urease-positive strains show a pink color, indicating urea hydrolysis (Zhao *et al.*, 2019).

Characterization of bacterial phytohormone production (Indole-3-acetic acid, IAA)

IAA compounds were detected among the isolates by a modified spectrophotometric method described by Khan *et al.* (2014). Briefly, the isolates were grown at 30 °C to the exponential phase ($\sim 10^8$ cells/ml) in 50 ml

of LB containing 1 mg/ml tryptophan incubated under rotation in darkness. After centrifugation, 1 ml of each supernatant was reacted with 2 mL of Salkowski's test solution for 25 min. A positive reaction was manifested by the development of a pink color measured using a UV spectrophotometry at 530 nm. The concentration of IAA is deduced from a standard curve plotted using authentic IAA (Sigma-Aldrich, Germany). Three replicates are performed for each isolate.

Assessing of plant growth promoting ability on wheat and tomato

The germination percentages of wheat and tomato seeds, as well as seedling growth experiments, were monitored following the procedure of Kabir *et al.* (2023). Three bacterial isolates (EpB, EdB6, and EdB8), the best IAA producers in this study, were tested. First, they were grown in yeast peptone broth (Merck, Germany) for 48 hours at 30 °C. Then, the cell pellets (10^8 CFU/ml), obtained after centrifugation and washing with sterile phosphate buffer (PB, 60 mM), were used to inoculate sterilized seeds. The assays were carried out in Petri dishes lined with sterile tissue paper in triplicate. Control seeds, without bacterial inoculation, were treated with PB (60 mM, pH = 6.8). The germination rate was determined after 8 days. The length of the roots and shoots was also recorded. The vigor index (VI) was computed using the formula $VI = [\text{mean shoot length (cm)} + \text{mean root length}] \times \text{germination\%}$.

Statistical analysis

The obtained results were assessed by the one-way analysis of variance (ANOVA) and Tukey's test. SPSS software version 25.0 was used, and treatment means were compared at significance level of $p < 0.05$.

Results

Isolation and enzymatic profile of bacteria

Eight bacterial strains (3 epiphytes and 5 endophytes) were identified with regard of their efficacy for enzymatic and phytohormone production. As shown in Table 1, the most strains (62.6%) were identified as *Bacillus*. Only two profiles were recorded within the Gram-negative group and matched well with the species *Bulkhordia cepacia* and *Pseudomonas luteola*. The profile EpB2 included the only coccobacilli identified as *Rhodococcus* spp. All isolates were able to produce amylase and lipase enzymes (Table 2). Seventy-five percent (6/8) of the isolates produced protease. There was evidence of esterase

activity in 37.5% (3/8) of the bacterial isolates. Only 25% (2/8) of the isolates could produce urease and gelatinase. Out of all the strains, EpB1 and EdB8 have exhibited remarkable extracellular enzymatic activity, and they correspond to the *Bacillus* (Table 2).

Table 1. Structural and biochemical traits of the bacterial epiphytes and endophytes recovered from the roots of *Origanum vulgare* L.

Test	EpB1	EpB2	EpB3	EdB4	EdB5	EdB6	EdB7	EdB8
Color	Cream	Off-White	Yellow	White-yellow	Cream	Yellow	Yellow	White-yellow
Margin	Undulate	Undulate	Regular	Regular	Undulate	Regular	Regular	Regular
Gram	+	+	-	+	+	-	+	+
Shape	Endo-spore forming rods	Cocco-bacilli	Rods	Endo-spore forming rods	Endo-spore forming rods	Rods	Endo-spore forming rods	Endo-spore forming rods
Catalase	+	+	+	+	+	+	+	+
Oxydase	-	-	+	+	-	-	+	+
Indole	-	-	-	-	-	-	-	-
Methyl red test	-	-	-	-	-	-	-	-
VP test	+	+	+	+	+	-	+	+
Glucose	+	+	+	+	+	-	+	+
Mannose	-	+	+	+	-	-	-	+
Inositol	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	-	+	-
Rhamnose	-	+	+	+	-	-	+	+
Sucrose	+	+	-	-	-	-	+	+
Melibiose	-	-	-	-	-	-	+	-
Amygdaline	-	-	+	-	-	-	-	-
Arabinose	-	+	+	+	-	-	-	+
Mannitol	-	+	+	+	-	+	-	+
Maltose	+	+	+	+	+	-	+	+
Cultivation at 4 °C	+	-	-	-	-	-	-	-
Cultivation at 25 °C	+	+	+	+	+	+	+	+
Cultivation at 37 °C	+	+	+	+	+	+	+	+
Cultivation at 44 °C	+	-	-	+	-	-	-	+
Identification results	<i>Bacillus cereus</i>	<i>Rhodococcus</i> spp.	<i>Burkholderia cepacia</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Pseudomonas luteola</i>	<i>Bacillus</i> spp.	<i>Bacillus subtilis</i>

+/: Functional trait present, -/: Functional trait absent

Table 2. Enzymatic characteristics of bacterial epiphytes and endophytes associated with the roots of *Origanum vulgare* L.

Enzyme	Amylase	Protease	Esterase	Lipase	Urease	Gelatinase
EpB1	+++	+++	+++	+++	–	+++
EpB2	++	–	–	++	–	–
EpB3	+	–	++	++	–	++
EdB4	+	+	–	++	–	–
EdB5	++	+	–	+	–	–
EdB6	+++	++	–	+++	P	–
EdB7	+	++	–	++	–	–
EdB8	+++	+++	+++	+++	P	–

P/: Functional trait present, -: Functional trait absent, +/: clear halo<5 mm, ++/: clear halo >5 mm, +++/: clear halo <8 mm

Characterization of bacterial phytohormone production

Varying levels of IAA were recorded in all eight isolates ($p < 0.01$) (Figure 2). The amount of IAA produced by endophytes ranged from 11.19 ± 0.1 to 114.1 ± 0.4 $\mu\text{g/ml}$, while epiphytes produced between 67.13 ± 0.2 and 114.56 ± 0.3 $\mu\text{g/ml}$. *Bacillus cereus* EpB1 was registered with the highest IAA concentration, followed by *Bacillus subtilis* EdB8, and *Pseudomonas luteola* EdB6.

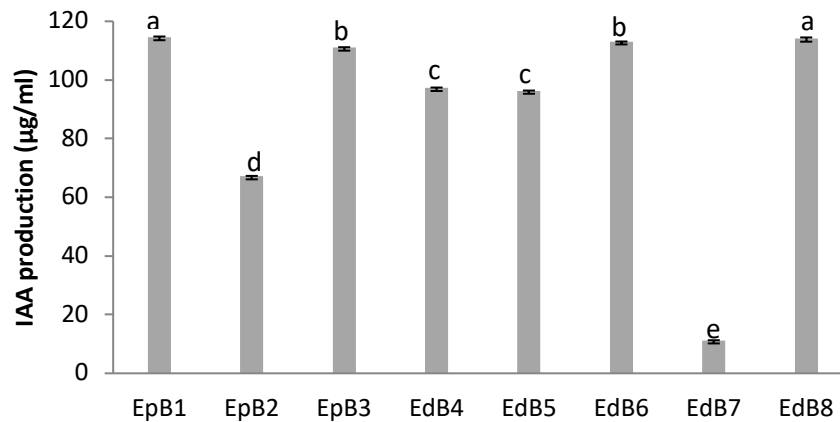


Figure 2. Indole-3-acetic acid production by epiphytic and endophytic root isolates of *Origanum vulgare* L. a, b, c, d, e/ indicate significant differences between the isolates (Tukey's test, $p < 0.05$).

Assessing of plant growth promoting ability on wheat and tomato

Compared to the uninoculated seeds, the three bacterial isolates selected (EpB1, EdB6, and EdB8) showed significantly ($p < 0.001$) better germination rate, seedling weight, and growth (Figure 3). The highest germination rate and vigor index of wheat were recorded in the isolate *Bacillus subtilis* EdB8. For tomato seeds, the *Bacillus cereus* EpB1 treatment gave the best germination, while EdB8 was the most effective for increases in vigor index. Overall, clear increases in fresh biomass, as well as in shoot and root lengths, were observed in the treated seeds. The strains *Bacillus subtilis* EdB8 and *Bacillus cereus* EpB1 showed the most significant improvements in shoot and root lengths in wheat. However, *Bacillus cereus* EpB1 resulted in the highest fresh and dry weights in wheat, while *Bacillus subtilis* EdB8 recorded the highest values in tomato (Table 3).

Table 3. Effect of bacterial inoculation of *Bacillus cereus* EpB1, *Pseudomonas luteola* EdB6, and *Bacillus subtilis* EdB8 on wheat and tomato germination rate and seedling growth measurements (means $\pm$ SE)

Parameters	Wheat seeds				Tomato seeds			
	Control	Treatment			Control	Treatment		
		EpB1	EdB6	EdB8		EpB1	EdB6	EdB8
Germination (%)	44.89 $\pm$ 5.13 ^a	84.61 $\pm$ 3.85 ^b	83.33 $\pm$ 4.43 ^b	93.59 $\pm$ 2.21 ^c	46.36 $\pm$ 2.14 ^a	88.74 $\pm$ 2.09 ^b	86.76 $\pm$ 2.07 ^b	88.51 $\pm$ 6.7 ^b
Vigor index	555.68 $\pm$ 4.1 ^a	2596.38 $\pm$ 23.4 ^d	2360.83 $\pm$ 5.5 ^c	2785.22 $\pm$ 4.7 ^d	328.34 $\pm$ 1.7 ^b	1082.63 $\pm$ 3.5 ^e	1257.02 $\pm$ 7.1 ^f	1273.92 $\pm$ 9.1 ^f
Shoot length (cm)	4.13 $\pm$ 1.55 ^a	14.50 $\pm$ 1.08 ^c	14.88 $\pm$ 0.85 ^c	15.33 $\pm$ 0.54 ^d	4.85 $\pm$ 0.62 ^a	6.25 $\pm$ 0.65 ^b	7.00 $\pm$ 0.91 ^b	8.75 $\pm$ 0.65 ^b
Root length (cm)	8.25 $\pm$ 0.65 ^a	16.18 $\pm$ 1.61 ^d	13.45 $\pm$ 0.42 ^c	14.43 $\pm$ 0.43 ^d	2.23 $\pm$ 0.17 ^b	5.95 $\pm$ 0.76 ^c	7.50 $\pm$ 1.08 ^c	5.63 $\pm$ 1.11 ^c
Shoot fresh weight (mg)	9 $\pm$ 0.02 ^a	350 $\pm$ 0.10 ^e	170 $\pm$ 0.02 ^c	110 $\pm$ 0.02 ^d	3 $\pm$ 0.01 ^b	14 $\pm$ 0.01 ^b	24 $\pm$ 0.01 ^c	35 $\pm$ 0.01 ^d
Root fresh weight (mg)	2 $\pm$ 0.004 ^a	213 $\pm$ 0.01 ^e	115 $\pm$ 0.01 ^c	125 $\pm$ 0.01 ^d	2 $\pm$ 0.06 ^a	40 $\pm$ 0.02 ^b	39 $\pm$ 0.06 ^b	48 $\pm$ 0.08 ^c
Shoot dry weight (mg)	0.9 $\pm$ 0.03 ^a	25 $\pm$ 0.13 ^e	11 $\pm$ 0.12 ^c	10 $\pm$ 0.06 ^b	0.5 $\pm$ 0.02 ^a	1.7 $\pm$ 0.03 ^b	2.9 $\pm$ 0.01 ^c	3.8 $\pm$ 0.02 ^d
Root dry weight (mg)	0.4 $\pm$ 0.01 ^a	23 $\pm$ 0.03 ^e	15 $\pm$ 0.02 ^c	18 $\pm$ 0.04 ^d	0.2 $\pm$ 0.03 ^b	5 $\pm$ 0.02 ^c	4 $\pm$ 0.03 ^c	5 $\pm$ 0.1 ^c

a,b,c,d,e,f/: Significant differences between values within the same line (Tukey's test; $p \leq 0.05$).

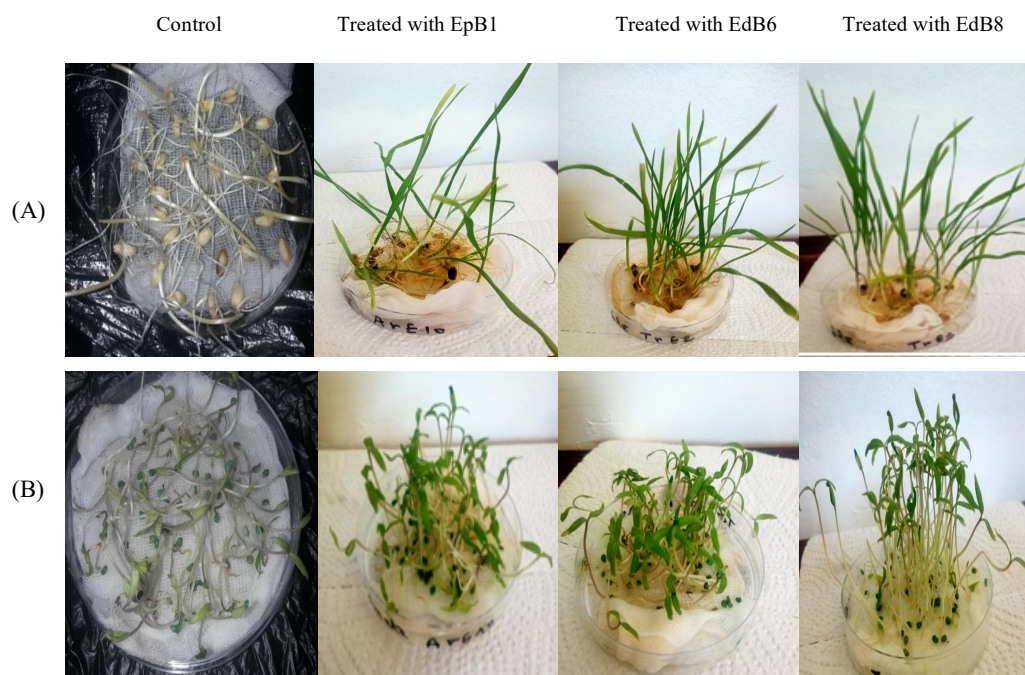


Figure 1. Plant growth promoting capacity of isolates on (A) wheat and (B) tomato seeds after 8 days

Discussion

Findings of this study revealed eight root bacteria from *O. vulgare*, identified as *Bacillus cereus*, *Rhodococcus* spp., *Burkholderia cepacia*, *Bacillus subtilis*, *Pseudomonas luteola*, and *Bacillus* spp., which showed various enzymatic properties potentially useful. Similarly, Bafana (2013) identified most of the genera found in the present research from *O. vulgare* roots in the sub-Himalayan region of Kangra Valley. He showed that these bacteria performed well under biological stress by producing several lytic enzymes and bioactive molecules. Both tested strains, *Bacillus cereus* EpB1 and *Bacillus subtilis* EdB8, showed remarkable enzymatic activity. *Bacillus* was previously known to be root-associated abundant bacteria of several medicinal plants like *Prosopis laevigata*, *Spharealcea angustifolia*, and *Aleo vera*, and this bacterium has also shown potential for the synthesis of cellulases, amylases, proteases, and xylanases (Roman-Ponce *et al.*, 2017; Silva *et al.*, 2020). Similarly, Hassan (2017) and Duhan *et al.* (2020) recorded high enzymatic activity in the *Bacillus* genus isolated from medicinal plants. Significant enzymatic activity was also observed among the endophytic isolate *Pseudomonas luteola*. *Pseudomonas luteola* has been isolated from different environmental samples, and some

strains have multiple traits of plant growth-promoting bacteria, which function as an efficient biofertilizer (Kurek *et al.*, 2013). It is relevant to mention that all isolates exhibited amylolytic and lipolytic activities, and the majority had proteolytic activity. Similar experiments on bacterial isolated from various medicinal plants are in line with the present data (Silva *et al.*, 2020; Abdel-Hamid *et al.*, 2021). Production of amylase and protease by epiphytic and endophytic bacteria is a significant pathway to enhance plant development; the plant's nutrient state can be improved by these enzymes. Furthermore, they exhibit bioactivity, which can contribute as a defense mechanism against pathogenic microorganisms for the host plant. Currently, endophyte enzymes such as amylase and protease constitute interesting compounds in industrial biotechnology because of their efficient role in biodegradation and biocatalysis (Fouda *et al.*, 2015; Hassan, 2017). However, microbial lipases have attracted attention for their insecticidal effect in degrading insect outer cuticle lipids, making their role in biological control highly innovative (Sicuaia *et al.*, 2015). Only *Bacillus cereus* EpB1 and *Burkholderia cepacia* EpB3 epiphytes strains had the capacity for gelatin liquefaction. Comparable observations were reported for gelatinase produced in *Bacillus cereus* (Sicuaia *et al.*, 2015) and *Burkholderia cepacia* (Mengxing *et al.*, 2021). Bacteria that degrade gelatin, a compound currently used as a biostimulant for plants, need to be studied as potential candidates for facilitating the degradation of gelatin into easily assimilated metabolites and consequently the growth and health of plants (Costa *et al.*, 2023).

The data analysis revealed significant differences in IAA synthesis between various epiphytic and endophytic bacterial strains in the presence of tryptophan ($p < 0.01$). The IAA is a secondary metabolite, and its synthesis by bacteria is influenced by the process by which the precursor tryptophan is utilized, environmental factors, growth rates, and nutrient accessibility (Herlina *et al.*, 2017). The present findings revealed that the highest IAA concentrations were registered for *Bacillus cereus* EpB1, *Bacillus subtilis* EdB8, and *Pseudomonas luteola* EdB6; suggesting that these bacteria exhibited the ability to improve the plant development. According to previous research, *Bacillus* and *Pseudomonas* were among the leading IAA-producing genera that stimulated plant growth (Bensidhoum *et al.*, 2016; Etminani and Harighi, 2018). In line with our findings, Hassan (2017) found that *Bacillus cereus* strains (Tp.1B and Tp.6B) selected from the medicinal plant *Teucrium polium* L. were the highest bacterial IAA producers. Ji *et al.* (2014) showed comparable results; they observed that the isolate *Bacillus subtilis* was a notable IAA producer and exhibited a significant ability to promote crop yield (wheat and maize). In the same way, Kurek *et al.* (2013) detected high in vitro IAA production by

Pseudomonas luteola in the presence of 500 µg/ml of tryptophan. The maximum IAA values recorded in the present study were remarkably high compared with those reported in the endophytes derived from other plant species including *Hyptis marrubioides* (95.13 µg/ml) (Vitorino *et al.*, 2012) and *Phyllanthus amarus* (1.4727 µg/ml) (Joe *et al.*, 2016). In contrast, they were lower than those observed for bacteria obtained from *Aloe vera* (225.2 µg/ml) (Silva *et al.*, 2020) and *Camellia sinensis* var. *assamica* (367.59 µg/ml) (Kabir *et al.*, 2023).

The three representative bacterial isolates (EpB1, EdB6, and EdB8), based on their maximum capacity to produce the plant hormone IAA, showed a significant effect on the germination rate, the vigor index, and the growth of wheat and tomato seedlings. These improvements exhibit resemblance to those obtained in other previous reports, which also reported the positive effect of treating wheat seeds with *Bacillus* strains (Rana *et al.*, 2011; Ansari *et al.*, 2019). Similarly, Malfanova *et al.* (2011) showed that the fresh weight of radish roots was 46% higher than the control when plants were treated with the endophytic strain *Bacillus subtilis* HC8. Krimi *et al.* (2016) reported that several tomato plant growth parameters, in particular seed germination, root length, fresh weight, and dry weight, were highly enhanced by inoculating seeds with endophytic species of the *Bacillus* genus, including *Bacillus cereus*. In the present study, *Pseudomonas luteola* EdB6 also had promising positive impacts on tomato seedling height. In a similar experiment, Widnyana and Javandira (2016) observed that inoculated tomato seeds with *Pseudomonas* spp. had a very significant height and number of leaves compared to untreated controls. According to the study by Dordevic *et al.* (2017), the improved growth parameters reported in this study seem to be linked to the IAA production highlighted, which is a regulator of early plant development such as elongation and branching of roots and shoots. Similarly, Ahmed and Hasnain (2014) demonstrated that the improvement in several plant part lengths is linked to the bacterial biosynthesis of IAA. The predicted mechanism for auxin's effect on cell elongation involves hormone-induced opening of calcium channels in the plasmalemma, which may result in modifications in calcium homeostasis in the cytosol (Dordevic *et al.*, 2017).

The biochemical tests revealed also that the EpB1 and EdB8 isolates produce acetoin, which function as an activator of induced systemic resistance (Ji *et al.*, 2014). Hence, we suggested that EpB1 and EdB8 strains can be a beneficial bioagents that stimulate resistance in plants. Several studies have also confirmed that treatment with PGP *Bacillus* strains induces antioxidant systems and energy metabolism in tomato and wheat roots, leading to increased plant growth and photosynthesis (Akram *et al.*, 2019; Choudhary *et al.*, 2021).

The data from this research suggested that the three isolates, EpB1, EdB6, and EdB8, can prove efficient and advantageous in agronomy and biotechnology practices, notably promoting plant development in wheat and tomato seedlings. However, more research is needed to evaluate their synergistic interactions, their colonization potential in different plants, and their competitiveness under field conditions.

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Conflicts of interest

The authors declare no conflict of interest.

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