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Research Article

Plant Growth Promotion and Biocontrol Potentiality of Endophytes Isolated from Root Nodules of *Sulla flexuosa* L. Plants

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Legumes, native to the Mediterranean, harbor reservoirs of endophytes that help plants adapt to various environmental stresses. The current study was carried out to evaluate the plant growth characteristics and antifungal activity of root nodule endophytes as biocontrol agents and plant growth promoters. Eleven bacterial endophytes isolated from root nodules of *Sulla flexuosa* L. grown in Northwest Morocco were assessed for their plant growth-promoting (PGP), and antifungal properties. Four endophytic bacteria were selected for their efficiency in solubilizing inorganic phosphate. The selected strains were positive for more than 2 PGP traits, including indole acetic acid, ACC deaminase, siderophore, and ammonia production. The screening for lytic enzyme production revealed that all strains were capable of producing chitinase, cellulase, catalase, and protease, while the secretion of amylase and urease was not detected. The HFB11 was the only strain incapable of producing pectinase. *In vitro* experiments revealed the strains' potential to withstand salt and drought stresses by being able to grow in high concentrations of NaCl and PEG. Based on 16S rRNA gene sequencing, the strains were identified as *Enterobacter* and *Serratia*. The antagonistic activity of the strains against *Botrytis cinerea*, *Aspergillus ochraceus*, and *Fusarium oxysporum* was detected and they were shown to inhibit the fungal growth with various percentages. The highest percentage of inhibition was observed for HFB3 against *B. cinerea* with 50% inhibition followed by HFB8 which was able to inhibit 47% of *F. oxysporum*'s growth. In contrast, a weak inhibition was observed against *A. ochraceus*. All these findings indicate that the chosen endophytes, halotolerant *Serratia inhibens* HFB8 and *Enterobacter hormaechei* HFB11, might be used as candidates for effective biocontrol and growth promotion of legumes.

1. Introduction

The Mediterranean Basin supports a diversified ecosystem of pasture legumes; however, recent research indicates that the Mediterranean area is particularly sensitive to climate change, with significant decreases in overall precipitation and increases in mean annual temperatures, soil mineral deficiencies, and/or toxicities [1]. Additionally, toxigenic soil fungal pathogens such as *Fusarium* sp., *Aspergillus* sp., and *Botrytis* sp., which are the most virulent fungi prevalent in the Mediterranean ecosystems due to their potential to survive on infested residues, can cause enormous diseases and losses in production cost in agroecosystems [2]. Nevertheless, a variety of legume species have adapted to growing in stressful environments, including *Hedysarum* spp. (*Sulla flexuosa* L.), a naturally occurring plant in Northern Morocco noted for its high fodder value and capacity to prevent soil erosion due to its deep and robust root structure [3]. The benefits of efficient symbiosis between legumes and nitrogen-fixing bacteria in agricultural output are widely known. They can fix substantial amounts of atmospheric nitrogen, allowing them to live in nitrogen-depleted soils without N fertilizers, while also maintaining higher nitrogen content in the soil. In addition to rhizobia, a variety of endophytic bacteria belonging to different bacterial genera such as *Bacillus*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Serratia*, and *Pantoea* have been widely reported to originate from different legumes such as alfalfa, soybean, chickpea, and peanut [4–9]. Microbial endophytes are a special group of soil microorganisms that

successfully colonize the root systems of their host plants and could effectively support plant growth [10]. Unlike rhizobacteria, endophytes form symbiotic relationships with many plants, flourish within the internal tissues of plants, and use specialized mechanisms to infiltrate the host [11]. Although the host mostly reaches the rhizosphere soil surrounding the roots, it is, however, important to note that this infiltration is not beyond the control of the host plant [12]. Numerous reports have highlighted the advantageous effects of endophytic bacteria in promoting plant growth directly and/or indirectly, regardless of the disadvantageous conditions. Endophytic bacteria are beneficial to the host plants due to several factors including, their ability to increase the intake of nutrients such as phosphorus or nitrogen, their production of phytohormones implicated in root growth, such as auxin and cytokinin, their involvement in development and biomass, in addition to inducing resistance to abiotic stresses [13–16]. They can also protect plants from pathogens by producing bioactive secondary metabolites, notably lytic enzymes, or by competing for nutrients via siderophores, which chelates the iron present in the soil, rendering it unavailable to pathogenic microorganisms [17]. The search for sustainable alternatives to enhance plant defense mechanisms is tempting. The use of endophytic bacteria as biocontrol agents offers an attractive alternative solution for tackling pathogen-related crop losses. There is a growing interest in using endophytic bacteria as bioinoculant agents to promote plant health and crop productivity, as they can be employed as biofertilizers, seed treatments, or foliar sprays [18]. This study, therefore, was carried out to isolate and identify root endophytic bacteria from *Sulla flexuosa* along with determining their multiple plant growth-promoting traits, tolerance to drought and salt stress, and antagonistic activity.

2. Materials and Methods

2.1. Sample Collection. Root nodule samples of *Sulla flexuosa* plants wildly grown in Boukhalef province of Tangier, Morocco (35°728240′N, 5°873565′W), were collected aseptically in sterile cotton swabs and contained in screw cap plastic tubes containing silica gel as described by Vincent [19], all sample tubes were stored at 4°C before being brought to the laboratory and processed for isolation. The soil sample analysis at the National Center of Scientific and Technical Research (CNRST) in Rabat, Morocco, revealed a clay texture with a low level of N, P_2O_5, and OM and an alkaline pH of 7.9 (Table 1).

2.2. Bacterial Strain Isolation. Healthy nodule samples were collected from the roots of *Sulla flexuosa*. The endophytic bacterial strains were isolated from surface-sterilized nodules as described by Vincent [19]. To guarantee purity, single colonies were picked and streaked repeatedly on Yeast extract mannitol (YEM) medium containing Congo red. The isolates were stored at −20°C in 25% (v/v) sterile glycerol. Successful surface sterilization of nodules was assessed by inoculating the water of the final rinse into YEM medium agar for 7 days at 30°C.

2.3. In Vitro Plant Growth Promoting Activities. The isolated strains were tested for PGP attributes *in vitro*, including their capacity to solubilize tricalcium phosphate, produce hydrogen cyanide (HCN), indole-3-acetic acid (IAA), siderophore, ACC deaminase, and ammonia.

2.3.1. Inorganic Phosphate Solubilization. The endophyte bacterial colonies were streaked into Pikovskaya medium (PVK) [20] to test their ability to dissolve Tri-Calcium Phosphate (TCP). The plates were incubated for 7 days at 28 ± 2°C, and colonies with a clear halo were considered Phosphate Solubilizing Bacteria (PSB). The diameters created by the bacterial colonies along with the clear zone were measured and used to determine the Phosphate Solubilization Index (PSI) using the following equation [21]:

$$\text{Phosphate solubilization index} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}. \quad (1)$$

The PSBs were inoculated in 50 mL of PVK broth, with uninoculated broth serving as control. The solubilized phosphorus was calculated using the Ames method [22] and the pH was measured using a pH meter (pH-221 Biobase).

2.3.2. Hydrogen Cyanide (HCN) Production. HCN secretion was qualitatively analyzed according to the method described by Bakker and Schippers [23]. The tested strains were streaked on a YEM medium containing 4.4 g·L^{-1} of glycine. A piece of Whatman filter paper No. 2 was soaked in a 0.5% solution of picric acid and placed on top of the plates. When exposed to HCN gas, the color of the paper changed from orange to brown.

2.3.3. Indole Acetic Acid (IAA) Production. Gordon and Weber's coulometric approach to investigate tryptophan-dependent IAA production was adopted [24]. Bacterial isolates were grown in Sucrose Minimal Salts (SMS) medium containing 0.05% of tryptophan. After two days of incubation at 28°C, the culture was centrifuged for three minutes at 13000 rpm. 1 mL supernatant was supplemented with 2 mL of Salkowski reagent. Absorbance of the test

Table 1: Physical and chemical soil analysis.

Boukhalif	Chemical composition							Physical composition				
	Water content %	pH	N %	P_2O_5 ppm	K_2O ppm	OM %	Total $CaCO_3$	Clay %	Fine silt %	Coarse silt %	Fine sand %	Coarse sand %
	5.2	7.9	0.146	13.16	147.57	1.38	18.19	63.83	13.3	0.09	1.49	1.97

mixture was taken at 535 nm after 20 minutes of incubation at room temperature.

2.3.4. Siderophores Production. The Schwyn and Neiland assay was followed to determine the synthesis of siderophores by the selected endophytes [25]. After incubating the CAS solution and culture bacteria in the dark for 30 min, the siderophore production was measured at 630 nm and computed. The formula proposed by Pal and Gokarn [26] was adopted to calculate the percentage of siderophores in units:

$$\%\ \text{Siderophores units} = \frac{\text{Ar} - \text{As}}{\text{Ar}} \times 100, \quad (2)$$

where, Ar = OD of reference (CAS reagent); As = OD of the sample.

2.3.5. ACC Deaminase Production. The presence of ACC deaminase was determined by the ability of the selected bacterial strains to use ACC as the sole source of nitrogen. According to the Jacobson et al. method [27], we compared the growth rates of bacterial strains cultured in the presence of two different nitrogen sources: ACC and ammonium sulfate $(NH_4)_2SO_4$, as well as a mineral source, magnesium sulfate ($MgSO_4{\cdot}7H_2O$). After 48 hours, the optical density at 600 nm was measured. Isolates with an OD greater than that of the $MgSO_4{\cdot}7H_2O$ solution were considered positive for ACC production.

2.3.6. Ammonia Production. The production of ammonia was tested by inoculating 10^6 UFC/mL of one-day-old culture into 10 mL of peptone broth and incubating at 28°C for 72 hours. After adding 0.5 mL of Nessler's reagent to each tube, a color change from yellow to brown indicated the production of NH_3 [28].

2.4. Extracellular Enzyme Production. The capacity of the bacterial isolates to degrade cellulose was tested by streaking the inoculant on cellulose, and Congo-Red agar media, as described by Gupta et al. [29]. Amylase activity was determined using a soluble starch-yeast extract medium [30, 31]. The clear zone in skimmed milk agar was used to determine the protease activity [32]. As for the determination of urease activity, the analysis was performed as described by Maheshwari et al. [33]. The pectinase production was determined as described by Cattelan et al. [34], while the appearance of a clear halo around the colonies on the pectin medium was employed as an indication of the presence of pectinolytic activity. Agar medium supplemented with colloidal chitin was used to screen for chitinase-producing strains, through the detection of clear zones around bacterial colonies on a cream background [35]. The catalase activity was examined as described by Dacre and Sharpe [36].

2.5. Molecular Characterization. Four BSP strains, HFB3, HFB7, HFB8, and HFB11, were identified based on 16S rRNA analysis using two universal primers, fd1 and rd1 [37]. Total DNA was extracted using the phenol/chloroform method [38] and adjusted to a final concentration of 100 $ng{\cdot}\mu L^{-1}$ using a Nanodrop spectrophotometer (Thermo Scientific™ NanoDrop 2000). The obtained sequences were assembled using the sequence alignment editing program Bioedit (7.0.5.3), checked manually, and compared with those from GenBank using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/blast.cgi/). Using Clustal W software, the sequence data were visually compared and aligned. The neighbor-joining method was used to generate phylogenetic trees while the bootstrap analysis used 1000 resamplings. The Molecular Evolutionary Genetics Analysis (MEGA) was used for all phylogenetic analyses.

2.6. Halotolerance Assay. The four selected endophytic strains were checked for salt and drought tolerance properties using YEM liquid medium supplemented with increasing concentrations of NaCl (*w/v*) (2.50%, 5%, 7.50%, and 10%) and polyethylene glycol PEG 6000 (*w/v*) (10% and 20%). The control plates consisted of 0.05% NaCl (*w/v*) and 0% PEG (*w/v*). Fresh bacterial cultures were used and incubated for 48 h at 28 ± 2°C, and the growth was assessed by OD_{600} measurements.

2.7. Antagonism Activity. The antifungal effects of endophytic isolates on the growth of *Aspergillus ochraceus* and *Fusarium oxysporum*, isolated and identified by El Aaraj et al. [39], as well as *Botrytis cinerea*, isolated from cultured strawberries in Larache, Morocco, were evaluated. The antifungal activity was tested by a dual culture assay [40]. A 5 mm disk of the tested fungal strain was placed in the center of plates containing Potato dextrose agar (PDA) medium, the bacterial cultures were streaked on both sides within a 30 mm distance from the fungal disc. Control experiments (without bacteria) were performed for each fungal strain. The plates were incubated at 25°C for 7 days and examined for mycelial growth inhibition. The inhibitory activity was defined using the following formula [41]:

$$\%\ \text{Inhibition of fungal growth} = \left(\frac{r1 - r2}{r1}\right) \times 100, \quad (3)$$

r1: Mycelial radial growth in the control test; r2: Mycelial radial growth of the treated plates.

2.8. Data Analysis. All experiments were performed in triplicate and the results were analyzed using ANOVA and Fisher's protected LSD test ($p < 0.05$) in Statgraphics Plus version 4.0.

3. Results

3.1. In Vitro Growth-Promoting Potential of Endophytes. Eleven endophytic bacteria were isolated from nodules of *Hedysarum* sp. Four isolates were selected based on their ability to solubilize TCP, which was confirmed by the presence of a clear zone surrounding the colonies on PVK agar medium. These isolates were further evaluated for their growth-promoting potential and biocontrol capacities. The screening results for the PGP characteristics are shown in Table 2. The selected isolates demonstrated important phosphate-solubilizing ability, with phosphate index solubilization ranging from 2.43 to 3.23. The quantitative estimation of TCP solubilization by the tested strains in PVK liquid medium was recorded. Two strains, HFB11 and HFB3 recorded the highest concentration needed for P solubilization (79.74 mg·L^{-1}), followed by HFB7 (68.94 mg·L^{-1}), while HFB8 reported the lowest value with a concentration of 52.58 mg·L^{-1}. The results illustrated in Table 2 regarding the available P and pH values were found to be inversely linked (negative correlation).

All of the selected endophytes were capable of producing high levels of IAA and siderophores in addition to their phosphate-solubilizing ability, however, no traces of HCN were detected with any of the tested strains. The amount of IAA produced in the presence of L-tryptophan as a precursor was very low, ranging from 0.27 mg·L^{-1} (HFB3) to 1.35 mg·L^{-1} (HFB8). As for siderophores production, the percentage ranged from 2.94% (HFB3) to 52.58% (HFB8). Moreover, HFB7, HFB8, and HFB11 were found to produce ACC deaminase upon adding ACC as the sole nitrogen source for bacteria. In terms of ammonia production, HFB3, HFB7, and HFB8 were the three strains that demonstrated a discoloration in peptone water from yellow to brown, therefore indicating the presence of a positive reaction.

3.2. Hydrolytic Enzymes. Regarding enzymatic activities, the screening revealed that all tested strains had the ability to produce chitinase, cellulase, catalase, and protease, but none could produce urease and amylase, while only one strain, HFB11, tested negative for pectinolytic activity (Table 2).

3.3. Molecular Identification Using 16S rRNA Gene Sequencing of Selected Endophytic Strains. The genetic characterization of the selected endophytic bacterial strains based on the nearly complete encoding of the 16S rRNA gene revealed a close relationship to Enterobacteriaceae family, inferring two genera, *Enterobacter* sp., and *Serratia* sp., with similarity values ranging from 95% to 100% (Figure 1). According to the phylogenetic tree inferred from the 16S rRNA gene sequences, the four strains isolated from *Sulla flexuosa* L. HFB3, HFB11, HFB8, and HFB7, were linked to *Enterobacter bugandensis* 247BMC^{T}, *Enterobacter hormaechei* subsp. *xiangfangensis* 10–17^{T}, *Serratia inhibens* S40^{T}, and *Serratia liquefaciens* ATCC 27592^{T} respectively. The sequences of these strains were deposed in NCBI GenBank under the following accession numbers HFB3 (OP316890), HFB11 (OP316893), HFB8 (OP316892) and HFB7 (OP316891).

3.4. Halotolerance Assay. The capacity of the chosen endophytic bacteria to grow in various concentrations of NaCl and PEG 6000 was investigated (Table 3). A drop in OD in conjunction with the increasing levels of NaCl and PEG was noticed, indicating the presence of various levels of stress tolerance. All tested isolates exhibited a halotolerant profile, with the exception of *Enterobacter bugandensis* HFB3 (Table 3). The ability of the strains to adapt to abiotic stresses revealed that three isolates, *Serratia grimesii* HFB7, *Serratia inhibens* HFB8, and *Enterobacter hormaechei* HFB11, were halotolerant with *Enterobacter hormaechei* HFB11 having the highest drought tolerance.

3.5. Antagonistic Activity. The *in vitro* screening revealed that the four strains can sufficiently disrupt the radial mycelial growth of *F. oxysporum* (16.67%–40%) and *B. cinerea* (35.56%–50%), while the sensitivity of *A. ochraceus* towards the selected bacteria appeared to be minimal in comparison, with inhibition percentages reported to be less than 20% (Table 4). Compared to other strains, *Serratia inhibens* HFB8 presented optimum antifungal efficacy against phytopathogens, effectively suppressing *F. oxysporum* and *B. cinerea*'s growth at a rate of 47% and 45%, respectively (Table 4).

4. Discussion

In this study, four endophytic bacterial strains were selected from a collection of eleven strains isolated from root nodules of *Sulla flexuosa* L., a Mediterranean forage plant with substantial ecological and economical significance. It is demonstrated that *Sulla flexuosa* harbors endophytic strains presenting numerous plant growth-promoting and antimicrobial abilities. The present study examined the inorganic phosphate solubilization, phytohormone IAA, siderophore, ACC deaminase, and ammonia production, in addition to extracellular enzyme secretion in order to select isolates displaying the most promising growth promotion and biocontrol properties of leguminous plants [42, 43]. The 16S rRNA sequencing confirmed the identity of these endophytic isolates. The four endophytic bacterial strains belonged to *Enterobacter* and *Serratia* genera, which are common species reported to be isolated from root nodules of various leguminous plants [7, 40]. Interactions between plants and endophytes have been reported to improve plant nutrition and to have a protective effect on the host plant

Table 2: Plant-growth promoting and enzymatic activities of the selected endophytes.

	PIS	HCN	IAA ($mg{\cdot}L^{-1}$)	Siderophores (%)	P ($mg{\cdot}L^{-1}$)	pH_f	ACC deaminase	Ammonia	Chitinase	Amylase	Cellulase	Protease	Urease	Pectinase	Catalase
HFB3	2.72^{d}	—	0.27^{a}	2.94^{a}	79.74^{c}	4.46^{ab}	+	−	+	−	+	+	−	+	+
HFB7	3.23^{c}	—	1.04^{b}	38.91^{c}	68.94^{b}	4.80^{b}	+	+	+	−	+	+	−	+	+
HFB8	2.43^{a}	—	1.35^{b}	52.58^{d}	52.14^{a}	3.88^{a}	+	+	+	−	+	+	−	+	+
HFB11	2.56^{b}	—	1.27^{b}	21.66^{b}	79.74^{c}	4.64^{b}	−	+	+	−	+	+	−	−	+

PIS, phosphate index solubilisation; IAA, Concentration of indole aceticacid; HCN, Hydrogen cyanide; ACC Deaminase, 1-aminocyclopropane-1-carboxylate deaminase; P, Concentration of solubilized P; +, Positive; −, Negative. The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different $p < 0.05$ (Fisher's least significant difference (LSD) test).

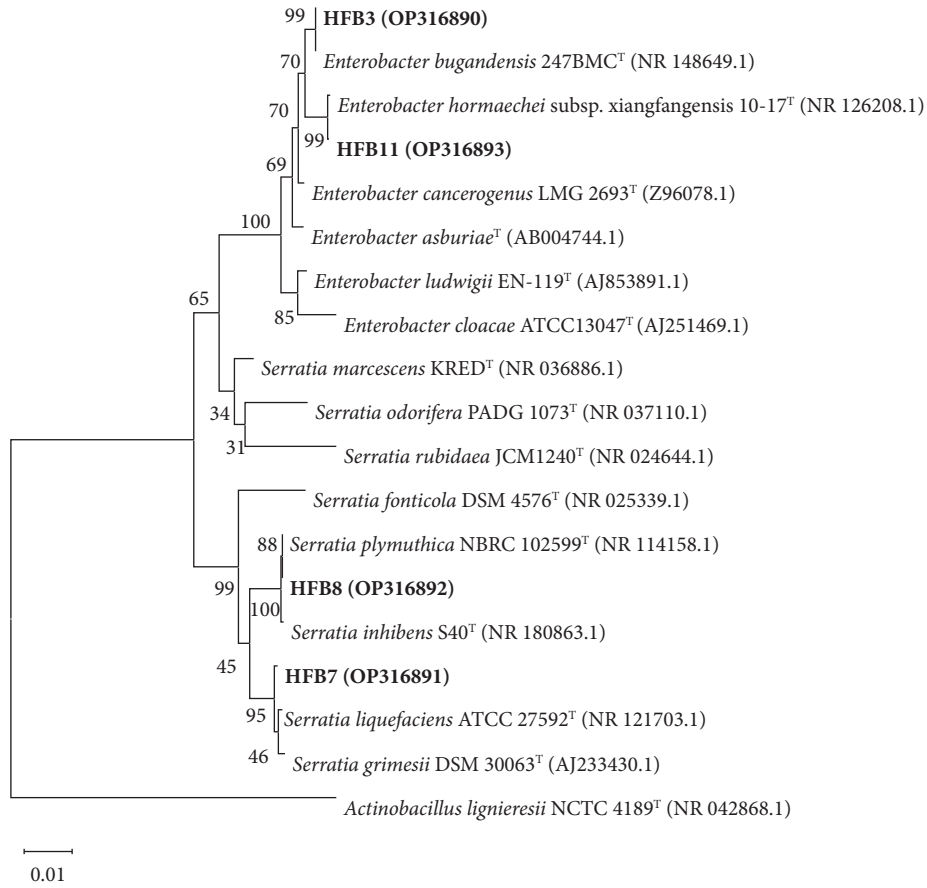


Figure 1: Phylogenetic tree of selected endophytes (denoted in bold) and their phylogenetically related species based on 16S rRNA sequences. Scale bar represents 0.01 substitutions per nucleotide position. The tree is rooted on *Actinobacillus lignieresii* NCTC 4189^T.

Table 3: Growth of endophytes under nonstressed (control), salinity and drought stressed conditions of different NaCl and PEG 6000 concentrations at OD_{600} nm.

Strains	Control	NaCl %			PEG 6000%	
		5	7.5	10	10	20
HFB3	0.671^{c}	0.572^{b}	0.308^{b}	0.145^{a}	0.530^{c}	0.214^{a}
HFB7	0.724^{b}	0.556^{ab}	0.373^{ab}	0.252^{c}	0.476^{a}	0.241^{b}
HFB8	0.866^{b}	0.613^{e}	0.337^{a}	0.235^{b}	0.513^{b}	0.238^{b}
HFB11	0.885^{a}	0.534^{a}	0.319^{c}	0.243^{bc}	0.468^{a}	0.349^{c}

The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different $p < 0.05$ (Fisher's least significant difference (LSD) test).

Table 4: Percentage of inhibitions of the endophytic bacteria studied on different phytopathogenic fungi.

	% Inhibitions of phytopathogenic fungi		
	Fusarium oxysporum	*Aspergillus ochraceus*	*Botrytis cinerea*
HFB3	16.67^{a}	19.17^{b}	50.00^{c}
HFB7	30^{b}	—	35.56^{a}
HFB8	47.5^{c}	2.5^{a}	45.93^{bc}
HFB11	40^{c}	19.17^{b}	41.48^{ab}

The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different $p < 0.05$ (Fisher's least significant difference (LSD) test).

against several biotic and abiotic stresses, they may boost host plant form by enhancing resistance to herbivores, heat, salt, diseases, and drought, as well as raising roots and leaves biomasses. Thus, endophytes can be considered as excellent host cohabitants [11, 41]. Phosphorus is considered a macronutrient that is required for multiple enzymatic activities widely implicated in numerous plant physiological processes. The data obtained in this study regarding endophytic bacteria revealed that the selected strains could boost phosphorus availability for plants through the solubilization of precipitated phosphates. They can equally improve soil phosphorus availability through different mechanisms, such as acidification, ion exchange, chelation, and organic acid synthesis, or by secreting acid phosphatase, which mineralizes organic phosphate [44]. The ability to generate IAA has a significant impact on plant growth and development, including root formation and proliferation, which improves water and nutrient uptake [45]. Endophytic bacteria that produce IAA, as is the case in this study, may directly boost plant development by increasing root surface area and length by promoting plant cell elongation or influencing cell division, allowing plants more access to soil nutrients. On the other hand, bacterial strains generating ACC deaminase have been shown to help plants cope with environmental stresses by promoting root and shoot growth and decreasing the inhibitory effects of ethylene secretion. The preliminary

analysis revealed that all endophytic bacterial strains were positive for ACC deaminase activity (Excluding *Enterobacter hormaechei* HFB11). Although siderophores may not be directly implicated in plant growth, these microbial-derived compounds can provide a source of iron and sustenance which can be used for growth stimulation [46]. Furthermore, siderophore-producing bacteria can inhibit the growth of competitive species by restricting the availability of Fe in the environment [47, 48]. The selected endophytes exhibited a capacity to produce a significant amount of siderophores that could be involved in boosting and protecting plants under stressed conditions. Numerous studies have reported the efficacy of plant growth-promoting endophytic bacteria to alleviate drought and salt stresses in plants [45, 46]. According to Kang et al. [49], inoculating alfalfa plants with drought-resistant *Enterobacter ludwigii* AFFR02 and *Bacillus megaterium* MJ1212 minimized the detrimental influences of drought stress, and increased plant growth and biomass content. The results obtained for the halotolerance assay indicated that the selected endophytic bacteria were able to grow in a NaCL concentration as high as 10% which is considered to be a higher tolerance rate than the one previously reported by Patel and Parekh [50] regarding *Serratia* sp. SG1 and *Enterobacter* sp. SRh isolated from *Salicornia brachiata* L., in this study, both strains were found to tolerate salinity levels up to 8% NaCL. Correspondingly, Mahgoub et al. [51] revealed that treating *Vicia faba* L. plants with native halotolerant endophytes *B. subtilis* AR5 and *B. thuringiensis* BR1 separately or in combination reduced the effect of salt stress and improved plant height, shoot dry weights, proline contents, enzyme activities, and mineral nutrient accumulation in shoot plants.

In regards to the strain's antagonistic effect, the endophytes *Serratia* HFB8 and *Enterobacter* HFB11 demonstrated strong antifungal activity in the current investigation, inhibiting over 40% of *Fusarium* sp. and *Botrytis* sp.'s mycelial growth. These findings contribute to the knowledge surrounding the well documented potential of strains such as *Bacillus*, *Pseudomonas*, *Streptomyces*, *Enterobacter*, and *Serratia* species as potential biocontrol agents against various plant pathogenic fungi [52]. The current study showed that bacterial strains with high biocontrol potential, *Serratia inhibens* HFB8 and *Enterobacter hormaechei* HFB11 are mutually capable of producing siderophores and cell wall-degrading enzymes, namely chitinases, cellulases, proteases, and catalases. The ability of *Serratia* sp., in particular, to produce its own chitinase has attracted the most attention in terms of its potential for biocontrol. In fact, it has been recognized by several studies as a biocontrol agent against *F. oxysporum*, *B. cinerea*, and *Rhizoctonia solani* [49, 50], which is in agreement with our findings. Further studies have been conducted that focused on the effect of *B. subtilis* on the reduction of the infection rate of *F. solani*, a phytopathogen widely responsible for root rot and boosting plant growth under salt stress, through an *in vitro* antagonistic screening of endophytic strains against fungal isolates from chickpea roots [53]. Hydrolytic enzymes could hydrolyze a variety of polymeric materials, including cellulose, hemicelluloses, chitin, proteins, and nucleic acid. The secretion of these enzymes may also improve the inhibitory activity against plant pathogens by dissolving their cell walls. Bacterial endophytes producing lytic enzymes are used as biocontrol agents to combat fungal and bacterial infections, as well as plant-parasitic nematodes [52, 54]. Overall, the antifungal capabilities of the studied potent endophytes could be accredited to a combination of factors such as competition for nutrients and space, synthesis of secondary metabolites, and production of lytic enzymes.

The current study demonstrated that *Serratia inhibens* HFB8 and *Enterobacter hormaechei* HFB11 both possess direct PGP activities such as inorganic phosphate solubilization, IAA secretion, siderophores production, and ACC deaminase production, as well as indirect PGP activities such as protease, cellulose, chitinase, catalase, pectinase, and antifungal activity. The utilization of advantageous soil bacteria to control plant diseases, a type of biological control, falls within the scope of green strategies. Similarly to our findings, *Serratia* and *Enterobacter* spp. are the most frequently reported for their biocontrol activity against phytopathogens [53, 55]. These PGPR Endophytic isolates have shown important properties that significantly promote plant growth, and therefore have proven their efficacy for potential application to alleviate abiotic and biotic stresses in plants, their application can supplementarily provide an eco-friendly and valuable alternative to chemical fertilizers. Since they are indigenous and competent in the rhizosphere, these PGP endophytes (HFB8 and HFB11) provide benefits to the host plant through interaction and metabolism with a high level of antagonistic potential against a variety of fungal phytopathogens. Thus, it could be proposed as a friendly microbiological agent without harming the environment.

5. Conclusion

The present study shows that root nodules of the Mediterranean native legume *Sulla flexuosa* harbors endophytic bacteria presenting at least two plant growth-promoting traits along with antimicrobial activity. The endophytes *Serratia inhibens* HFB8 and *Enterobacter hormaechei* HFB3 displayed a variety of plant growth-promoting properties which makes them strong contenders for bioinoculant production, thereby improving the health of the soil in an eco-friendly manner, all while increasing the population of Sulla plants, and effectively preventing them from becoming endangered species. However, further research into their PGP and biocontrol nature in the presence of plants is required to fully explore their biotechnological potential.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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