Bioprospecting Beneficial Endophytic Bacterial Communities Associated With Rosmarinus Officinalis for Alleviating Plant Productivity

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Abstract

The present study was aimed to isolate and identify root endophytic bacteria with multifunctional plant growth promoting (PGP) traits from medicinal plant *Rosemarinus officinalis* grown in the North-Western Himalayas. A total of 42 strains were isolated, exhibiting variable degrees of PGP traits, including P-solubilization (10-375 µg/ml), IAA (6-66 µg/ml), siderophore (32.37-301.48 %SU) production and antifungal activity in terms of percent growth inhibition (%GI) against *Fusarium oxysporum* (44.44-77.77 %GI), *Fusarium graminiarum* (48.88-71.42 %GI) and *Rhizoctonia solani* (44.44-77.7 %GI). 16S rDNA sequencing results showed lineage of these strains to 15 genera *viz., Aneurinibacillus*, *Bacillus*, *Beijerinckia*, *Cedecea, Ensifer*, *Enterobacter, Kosakonia*, *Lactobacillus*, *Lysobacter, Oxynema*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Pseudoxanthomonas* and *Serratia*. The effect of 11 potential strains was selected for *in vivo* growth studies of *R. officinalis*. The results showed that the inoculation of *Bacillus subtilis* KU21, *Pseudomonas aeruginosa* SI12, and *Cedecea lapagei* KU14 significantly increased the physical growth parameters of plant over uninoculated control *viz*., number of lateral of branches (43.95-46.39 %), stem height (29.04-38.57 %), root length (32.31-37.14 %), shoot (34.76-40.91 %) and root biomass (62.89-70.70 %). Physiological characteristics such as total chlorophyll (30.41-30.96 %), phenol (14.43-24.55 %) and carotenoids (34.26-39.87 %) content, also showed a relative increase as compared to uninoculated control; furthermore, the macronutrients (NPK) contents of the plant as well as soil also showed an increase. The developed module may be recommended for sustainable production of *R. officinalis* in the North-Western Himalayan region without hampering the soil health and fertility.

Introduction

Plants develop an intricate association with a variety of microorganisms including rhizospheric, and endophytic, which have attracted the attention of scientific community due to their verified benefits (Cordero et al. 2017; Liotti et al. 2018). These microorganisms are natural inhabitants of host plant and can also develop in an endogenous fashion. This is true for endophytic microorganisms, which colonize plant tissues for at least a part of their life-cycle without any visible disease symptoms (Silva et al. 2019). Bacteria of endophyte community are diverse and able to disperse throughout plant tissues systemically (Bacon and White 2016). Such colonization provides many advantages as this community, independent of its environmental circumstances, can interact effectively with the plant (Santoyo et al. 2016). This improves plant growth and a defense against plant pathogens, which further enhances stress tolerance, and can promote the synthesis or development of bioactive compounds of interest (Khamwan et al. 2018; McMullin et al. 2018).

Various studies have shown that roots associated bacterial strains can promote plant growth by mechanisms such as phytostimulation, biofertilization, and biocontrol (Gaiero et al. 2013; Abbamondi et al. 2016). Such association has direct physiological effects on plant growth and production, such as: nitrogen fixation, phosphate solubilization, production of ammonia, siderophores, phytohormones and hydrolytic enzymes (Silva et al. 2019). These effects provide for the need of plant nutrition in a sustainable manner, thereby reducing the use of chemical fertilizers and pesticides. This in turn preserves the soil biological diversity, thus providing an alternative to the conventional methods of cultivation and compelling the researchers to look for eco-friendly and sustainable agriculture production methods. To this end, the use of plant growth promoting rhizobacteria (PGPR) has emerged as an attractive approach in rosemary production. They are often used as plant growth enhancers under both normal and stressful conditions.

Medicinal plants are selective, while forming endophytic associations, since this choice may be based on the production of secondary metabolites and composition of root exudates; hence, a diverse group of bacterial communities exists based on their nutritional requirements, environment and soil type in which they are found (Maggini et al. 2018).

R. officinalis (rosemary) is a member of Labiatae family and is one of the most popular medicinal herbs rich in polyphenols (carnosic acid and rosamarinic acid) and flavanoids. It is native to Mediterranean region and cultivated worldwide in cool regions at elevations of 1000–3000 meter above sea level (masl) (semiarid and sub humid bioclimatic regions). In Himachal Pradesh, rosemary is grown at an elevation range of 1050–2100 masl falling in the mid-hills sub humid and high hills temperate wet zone. Owing to its numerous biological activities (antibacterial, antiproliferative, anti-inflammatory, and antioxidant), this plant has gained more interest from commercial point of view (Bourhia et al. 2019). However, to date the endosphere of rosemary has remained untapped and unexplored. Some studies have been carried out previously to evaluate the impact of commercially available PGPR on growth parameters and quality of bioactive compounds (flavonoids, phenolic acids, especially rosmarinic acid) of *R. officnalis* (Dehghani et al. 2019; Sadegh Kasmaei et al. 2019). So far, no published study has examined the PGP potential of native bacterial endophytes correlated to the growth of *R. officnalis* in North-Western Himalayas. The hypothesis underlying the present study is that the bacterial endophytes of this plant are promising bio-inoculants that can alleviate plant health. The outline of this study concentrates on assessment of functional diversity of culturable root bacterial endophytes of *R. officinalis* and *in vivo* studies of potential strains on the productivity of *R.officinalis* and soil functions.

Materials And Methods

Collection of root samples

Root samples of *R. officinalis* seedling were collected during the summer (June 2017) from rosemary cultivating sites (Kangra, Kullu, Solan, and Sirmour) of Himachal Pradesh. 2 year old plants (in vegetative state) were selected for sampling from all locations. Geographical coordinates and environmental conditions of sampling sites are presented in Table S1. A total of twenty-four (3 samples×8 sites) composite root samples were obtained from all the locations and stored in plastic bags at 4 ºC for further assaying of bacterial community structure.

Isolation of bacterial endophytes

Surface sterilization of roots was performed for isolation of bacterial endophytes following the standard method (de Favaro et al. 2012) with slight modification as follows: root samples were washed under running water, sterilized with 70 % ethanol for 45 sec, and 2 % sodium hypochlorite for 5 min followed by washing 4–5 times with sterile distilled water. The surface sterility of roots was cross-checked by plating 100 µl of the final wash and incubated

overnight at 30 ºC. No growth on plate indicated complete sterilization of roots. Furthermore, root endophytic bacteria were isolated using serial dilution spread plate technique. An aliquot of 100µl of suspension (10^{-2}-10^{-4} dilutions) was spread on nutrient and tryptic soy agar medium and incubated at 30 ºC till the appearance of bacterial colonies (upto 5 days). Isolated bacteria were enumerated as colony forming units per gram of roots (cfu g^{-1} root).

A total of 42 distinct morphotypes were selected and purified on respective medium. The pure culture of these strains was preserved on petri plates at 4 ºC for further analysis.

Morpho-biochemical characterization of endophytic bacteria

Microscopic examination of endophytic bacteria was done together with biochemical characterization according to Bergey's Mannual of Determinative Bacteriology (Holt et al. 1994). Antibiotic sensitivity test of isolates to standard antibiotics was evaluated using antibiotic sensitivity kits (Hexa universal 1, 2 and Hexa Pseudo 1,2) (Himedia, India).

Identification of endophytic bacteria

16S rDNA sequence analysis was employed for molecular identification of isolated bacteria. Genomic DNA was extracted using the conventional method (Sambrook et al. 1989) followed by PCR-mediated amplification with a set of universal primers (16SF: 5'- AGAGTTTGATCCTGGCTCAG-3'and16SF: 5'-AAGGAGGTGATCCAGCCGCA-3´). PCR reaction mix of 25 µl was prepared with 50 ng of template DNA, 20 pmol of each primer, 0.2 mM dNTPs, and 1U Taq polymerase in 1X PCR buffer. The reaction was cycled 35 times as denaturation at 94ºC for the 30s, annealing at 55ºC for 30 s and extension at 72ºC for 1 min 30 sec followed by a final extension at 72ºC for 10 min. The PCR product was analyzed by gel electrophoresis on 1.2 % (w/v) agarose gel. A band of ~ 1400bp was excised from the gel and purified using a gel extraction kit (RBCs Real Genomics, New Taipei City, Taiwan) and was sequenced by Genei labs (Bangaluru, India). Based on 16S rDNA sequences, phylogenetically related bacteria were aligned using a BLASTn search (Altschul et al. 1997). Multiple alignments with sequences of related taxa were implemented using CLUSTAL W (Higgins et al. 1994). A neighbor-joining phylogenetic tree was constructed with other 16S rDNA sequences of the related taxa retrieved from the GenBank database using MEGA X software.

In vitro screening for traits involved in plant growth promotion

Isolated bacteria were further authenticated by subsequent *in vitro* experiments to see whether they exhibited qualities which identified them as possible plant growth promoting bacterial endophytes (PGPBEs). Each *in vitro* screening test was conducted in triplicates. P-solubilization activity was determined by the method of Pikovskaya (1948). Quantitative production of IAA was estimated using the colorimetric method described by Gorden and Palleg (1957). To test the efficacy of endophytic bacteria as nitrogen fixers, loop full of 24 h old culture of each isolate were streaked on nitrogen free agar medium and incubated for 72 h at 30 °C. Colonies showing growth on inoculated medium after being transferred ten times in the same medium were potent nitrogen fixers (Jensen 1987). The ability of isolates to produce siderophore and hydrocyanic acid (HCN) was also assessed by Schwyn and Neilands (1987) and Bakker and Schipper (1987) methods, respectively. For lytic enzyme activity, spot inoculation was done on minimal agar medium amended with 0.3% colloidal chitin for chitinase (Robert and Selitrennikoff 1988), starch agar medium for amylase (Shaw et al. 1995) and skim milk agar plates for protease activity (Fleming et al. 1975). Ammonia production was observed according to the method of Cappuccino and Sherman (1992).

The antagonistic activity of the bacterial isolates against test fungal pathogen *viz., F. oxysporum* (ITCC 7337), *F. graminiarum* (ITCC 5334) and *R. solani* (ITCC 5308), was done by agar dual plate method on malt extract agar (MEA) medium and the percent growth inhibition (%GI) was calculated as described by Vincent (1947).

Stimulation of plant growth

To test the efficacy of endophytic strains for stimulating plant growth, a pot experiment was conducted. Eleven isolates with best *ex situ* PGP traits were selected. The potting mixture was prepared by mixing sand, soil and farm yard manure (FYM) in a ratio of 1:2:1. The following mixture was then subjected to intermittent sterilization i.e. three successive autoclave cycles of 1 h each at 100 ºC with 24 h of incubation between each cycle (tyndallization). The pH of potting mixture was determined in 1:2.5 (soil:water) suspension and the electrical conductivity (E.C.) of the supernatant liquid was recorded and expressed in dSm^{-1} (Jackson 1973). Furthermore, organic carbon (O.C.) was determined by chromic acid titration method of Walkley and Black (1934). Available N, P, and K contents of soil were determined following standard procedures (Tandon 2009).

The properties of the potting mixture were: pH 7.01; E.C. 0.69 dSm^{-1}; O.C. 1.12%; available N, P and K contents 290.32, 23.40 and 315.45 Kg ha^{-1}, respectively. The soil used for pot (20.00 cm diameter and 16.00 cm deep) experiment belongs to Entisols order as per United States department of Agriculture Soil Taxonomy. 4 kg of potting mixture was filled in the pots before commencement of experiment. Two months old seedlings of *R.officinalis* were procured from the Department of Forest Products, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, and were washed under running tap water to remove the soil adhering to the roots. Pure cultures of bacterial isolates were inoculated into 100 ml flask containing nutrient broth and incubated at 30 ºC for 24 h on a rotary shaker (MAC, Rotary Orbital Shaker, MSW-132) (at 120 rpm). Bacteria were subsequently pelleted by centrifugation at 6,000 rpm for 10 min. The pellets were washed with sterile distilled water three times, and the concentration of cells adjusted to 1× 10^8 cfu ml^{-1} by dilution. This liquid formulation was used as inoculum (Mortensen 1997). Roots of seedlings were immersed in prepared inoculum for about 1 h before plantation. Two seedlings per pot were planted and allowed to grow for four months. Booster doses of liquid bacterial cultures of the same cell density were applied at the rate of 20 ml $plant^{-1}$ with 15 days interval after planting. Seedlings were watered daily during the first two weeks of planting followed by irrigation once every two days. The following 12 treatments were arranged in a completely randomized block design (CRD) with three replications for each treatment: T1, control (uninoculated); T2, *Pseudomonas mediterranea* KA7; T3, *Pseudomonas oryzihabitans* KU5; T4, *Bacillus flexux* KA10; T5, *Pseudomonas aeruginosa* SI12; T6, *Pseudoxanthomonas japonensis* KU13; T7, *Pseudomonas putida* KU2; T8, *Cedecea lapagei* KU14; T9, *Pantoea agglomerans* KA14; T10, *Pseudomonas koreensis* KA11; T11, *Bacillus simplex* KA2; T12, *Bacillus subtilis* KU21.

Observations on plant growth parameters such as shoot and root length (cm), biomass (g), and the number of lateral branches per plant were recorded by following standard methods. Oven-dried plant samples were ground and sieved for the estimation of macronutrients (NPK). The total concentration of N in plant samples was determined using micro-Kjeldhal's method (Helrich 1990). Plant samples were digested in a diacid mixture of HNO_3:$HCLO_4$ (4:1) for P and K analysis (Jackson 1973). P concentration was tested in the digested sample (Jackson 1973). K in the digest was analyzed using the flame photometer (Biogen, Microcontroller Flame Photometer) (Jackson 1967). Total chlorophyll, carotenoids, and total phenol content (TPC) of leaf samples were determined using methods of Withem et al. (1971), Brezeanu et al. (2005), and Faust and Mikulewics (1967), respectively.

Endophytic nature of bacterial strains

To test whether the bacterial strains were capable of colonizing plant tissues, enumeration of the total endophytic bacterial population was done using standard serial dilution spread plate method after termination of the experiment. Colonies which showed morphological characteristics similar to treated strain were selected and the dominance of inoculated strain was calculated according to Simpson's index of dominance (D) as:

$D = Pi^2$

Where, Pi is the relative abundance of isolates calculated according to the following equation $Pi = ni/N$

n_i, is the number of inoculated strain colonies and N, is the total number of endophyte colonies

Statistical analysis

All the experiments were conducted under the statistical framework with three biological replications along with equal number of appropriate controls. The data obtained from the laboratory experiments and the net house was subjected to one-way analysis of variance (ANOVA) using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 7.0 (Microsoft, Redmond, WA, USA). The means and standard deviation of data were also calculated. Comparisons of treatment means were performed by the Fisher's Projected LSD (least significant differences) test at $P \leq 0.05$ level of significance. PCA (principal component analysis) was performed on pot experiment, to evaluate the relationship between effects of endophyte inoculation on several plant growth parameters. PCA was performed using PAST 3.0 software.

Results

Isolation and identification of endophytic bacteria

A total of 42 strains were isolated based on unique colony morphologies on medium used for isolation. Amongst these, 17 were Gram's positive while 25 were Gram's negative, varied from rods, cocci to coccobacilli. Endospore formation was observed in 16 isolates. Biochemically, low number of isolates were positive for indole (28.57 %) and hydrogen sulfide (H_2S) (9.52 %) production. Greater numbers of bacterial isolates were positive nitrate reduction (80.95 %) and oxidase production (61.90 %). All the isolates were positive for catalase except KU20 and KU25. 59.52 % were positive for methyl red, 40.47 % showed positive test for voges proskauer (VP); only 21.43 % bacterial isolates were able to hydrolyse gelatin. Almost all the isolates were able to ferment dextrose and sucrose, whereas a few isolates showed positive lactose fermentation test (Table S2).

Phylogenetic analysis based on 16S rDNA sequencing and alignment showed that these endophytes were affiliated to 2 phyla, i.e., Proteobacteria and Firmicutes, 15 genera *viz.*, *Aneurinibacillus*, *Bacillus*, *Beijerinckia*, *Cedecea, Ensifer*, *Enterobacter, Kosakonia*, *Lactobacillus*, *Lysobacter, Oxynema*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Pseudoxanthomonas* and *Serratia* and 32 species (Fig. 1A, 1B). The most dominant endophytic bacteria were reported in genera *Bacillus* and *Pseudomonas* representing 33.33 % and 25.64 % of the total isolates.

Beneficial Plant traits of bacterial strains

In vitro screening revealed that most of the strains exhibited multiple PGP activities. All the strains substantially solubilized mineral P in PVK broth (70–375 µg ml^{-1}). Majority of isolates (85.71 %) synthesized IAA (10–66 µg ml^{-1}) and produced siderophore (92.85 %) that ranged from 35.71-301.48 %SU. The ability to fix nitrogen on Jensen's nitrogen-free medium was recorded in 66.66% of strains. Strains exhibited potential beneficial traits belonging to genera *Bacillus, Cedecea*, *Enterobacter*, *Pantoea* and *Pseudomonas* (Fig. 2).

All the bacterial strains were able to produce one or more cell wall degrading enzymes. In total, 73.81 % strains exhibited chitinase activity, and 80.95 % were protease and amylase producers, thereby conferring antagonistic and endophytic properties to the selected strains. Majority of bacterial strains (88.09 %) displayed ammonia production, whereas; only 40.47 % were HCN producers. Endophytes belonging to genera *Aneurinibacillus*, *Bacillus*, *Cedecea*, *Pseudomonas* and *Paenibacillus* possessed efficient antagonistic traits of plant growth promotion (Fig. 2).

The antagonistic activity was observed for endophytic strains against phytopathogenic fungi (*F. oxysporum*, *F. graminearum* and *R. solani*). 40.47 % strains inhibited the growth of *F. oxysporum* (44.44–77.77 %GI), 33.33 % and 26.19 % strains were antagonist against *F. graminiarum* (48.88–71.42 %GI) and *R. solani* (44.44–77.77 %GI), respectively. Only six strains showed antagonism against all fungal pathogens, out of which SI12 strain was most effective against all the three tested fungal phytopathogens (Table 1).

Effect of endophytic bacteria on plant growth promotion

A pot experiment was conducted to validate the *in vitro* PGP activities of selected strains on the growth of *R. officinalis*. The *in vitro* PGP traits of selected strains have been depicted in Table 1.

Effect on physical characteristics

Most of the isolates significantly ($P \leq 0.05$) increased growth parameters of *R. officinalis* over uninoculated control. Treatments receiving *P. aeruginosa* SI12 (T5) inoculation showed maximum stimulatory effects for overall plant growth parameters over uninoculated control, which was statistically at par with the treatments receiving *C. lapagei* KU14 (T8) and *B. subtilis* KU21 (T12) inoculation (Table 2). These incremental effects were 43.95%, 45.61%, and 46.39% for number of lateral branches, 38.30%, 29.04% and 38.57% on stem height, 34.76%, 37.21% and 40.91% for shoot biomass, 62.89%, 70.70% and 63.29% for root biomass over untreated control.

Effect on physiological characteristics

Photosynthetic pigments of *R. officinalis* leaves were determined to evaluate the impact of endophytic strains on photosynthetic efficiency of host plant. Data corresponding to biochemical parameters revealed that treatment T5 (*P. aeruginosa* SI12) had maximum significant ($P \leq 0.05$) increase in total chlorophyll (33.09%), carotenoids (39.87%) and total phenol content (24.55%) of *R. officinalis* leaves over uninoculated control, which was at par with T8, T12 and T12 for chlorophyll and treatments T3, T7, T8, T11 and T12 in case of carotenoids (Fig. 3).

Principal component analysis of growth characteristics of R. officinalis in response to inoculation with endophytic bacteria

Principal component analysis of growth parameters of *R. officinalis* revealed that PC1 and PC2 accounted for 94.67 % and 3.93 % of the total data variation, respectively (Fig. 4). PC1 comprised treatments with T3 (*P. oryzihabitans* KU5), T6 (*P. japonensis* KU13), T7 (*P. putida* KU2), and T9 (*Pantoea agglomerans* KA14) showed a strong relationship with several lateral branches and stem height. While in PC2, treatments with T4 (*Bacillus flexus* KA10), T7 (*P. putida* KU2), and T9 (*Pantoea agglomerans* KA14) reported more influence on root biomass, total chlorophyll, and phenol content. This analysis showed that inoculation with potential plant growth-promoting bacterial endophytes had a significant effect on growth and productivity of *R. officinalis*.

Effect on plant nutrient concentration

The data appended in Fig. 5 illustrate that treatment (T12) receiving *B. subtilis* KU21 inoculation had maximum significant increase in N, P and K content by 33.33%, 61.54% and 54.54%, respectively over untreated control and was at par with T5 (*P. aeruginosa* SI12) and T8 (*Cedecea lapagei* KU14) for N content and with T8 (*Cedecea lapagei* KU14) for P content.

Effect on soil properties and endophytic population

None of the treatments influenced the soil pH, E.C. and O.C. significantly in comparison with the initial soil test values (data not shown) recorded before the trial whereas the contents of available nutrients (NPK) increased significantly by the sole application of endophytic strains (Table 3). Maximum significant increase in available NPK content (16.79 %, 36.26 % and 7.56 %) were recorded in treatment T12 (*B. subtilis* KU21) which was statistically at par with T8 (*C. lapagei* KU14) for N, T5 (*P. aeruginosa* SI12) for P and T11 (B. simplex **KA2)** for K content. None of the treatments influenced the soil pH, EC, OC significantly over soil initial test value (data not shown). Total endophytic bacterial count varied from 31.00 to 54×10^2 cfu g^{-1} root with the maximum count (54×10^2 cfu g^{-1} root) in *Bacillus subtilis* KU21 inoculated plants. Also, in the root endosphere of *B. subtilis* KU21 inoculated plants accounted for a maximum number (48×10^2 cfu g^{-1} root) of bacterial colonies matching *B. subtilis* KU21 with maximum Simpson's index of dominance (0.79).

Intrinsic antibiotic resistance of potential endophytic bacteria

B. subtilis KU21, *C. lapagei* KU14 and *P. aeruginosa* SI12 were evaluated for antibiotic sensitivity against combination of antimicrobial sensitivity discs (Table 4). It was observed that the tested strains were resistant to most of the antibiotics. But polymyxin B (300 µg) and colistin (10 µg) inhibited the growth of *Pseudomonas aeruginosa* SI12. *C. lapagei* KU21 was susceptible to neomycin (30 µg) and Co-trimoxazole (25 µg). Amikacin (30 µg) inhibited the growth of all three tested strains.

Discussion

Despite the great interest in plants used for the purpose of traditional medicine, little is known about the symbiotic associations of these plants with endophytic microorganisms (Silva et al. 2019). The present study is the first of its kind to evaluate the multifunctional potential of bacterial strains isolated from the roots of *R. officinalis* native to the North-Western Himalayan region of Himachal Pradesh, India. In this study, a collection of 42 root endophytic bacteria of *R. officinalis* was obtained from 4 different rosemary cultivating locations of Himachal Pradesh. These strains were identified using 16S rDNA sequencing and phylogenetic analysis. The isolates belonged to 15 genera and 32 species, mainly belonging to Proteobacteria and Firmicutes. These results confirmed rich endophytic pool in medicinal plants, in agreement with the previous studies (Elmagzob et al. 2019; Silva et al. 2019; Abdelshafy Mohamad et al. 2020). The predominant reported genera were *Pseudomonas* and *Bacillus*. Our findings are in line with the earlier studies which confirmed that *Bacillus* and *Pseudomonas* as PGPBEs have been widely found in medicinal plants such as *Pinellia ternate, Lycium Chinese, Digitalis pupurae*, (Miller et al. 2012); *Lonicera japonica* (Zhao et al. 2015); *Ginkgo biloba* (Yuan et al. 2012); *Clerodendrum colebrookianum* Walp. (Passari et al. 2016); *Thyme vulgaris* (Abdelshafy Mohamad et al. 2020).

The main purpose of the current study was to understand the interaction of endophytes with the host plant which involves mobilization of nutrients, production of phytohormones, siderophores, and antagonistic compounds. That is why bacterial strains were screened for *in vitro* traits of plant growth promotion with an aim to obtain potential candidates. These strains exhibited multifaceted PGP traits. Similar investigations reported that endophytic bacteria exhibited multiple traits of PGP (Egamberdieva et al. 2017). Among all, the strains of *Bacillus, Pseudomonas* and *Cedecea* exhibited the highest amounts of P-solubilization, siderophore, IAA, HCN, ammonia and lytic enzymes (chitinase, protease, and amylase). Earlier studies have also indicated that several *Bacillus* species isolated from medicinal plants produced phytohormones, solubilized P and improved growth of tomato (Abdelshafy Mohamad et al. 2020) and *Pseudowintera colorata* (Purushotham et al. 2020). Similarly, *Pseudomonas* species produced IAA and increased plant biomass of medicinal plant *Astragalus*

mongholicus (Sun et al. 2019). In contrast, few studies had reported PGP potential of *Cedecea*. For example Beniassa et al. (2019) reported the potential of *Cedecea* as P-solubilizer, nitrogen fixer and HCN producers under *in vitro* conditions.

In vitro screening for antifungal activity showed that six strains inhibited the growth of all tested fungal pathogens (*F. oxysporum, F. graminiarum*, and *R. solani*). These antagonistic strains exhibited various antifungal properties *viz*., siderophore, chitinase, protease, amylase, HCN, and ammonia production, etc. Thus, these endophytes can protect the plant from phytopathogenic fungi either by degrading the cell wall or by stimulating systemic resistance in plants (Mohamad 2018). Similar work has been carried out by Egamberdieva et al. (2017), and Liu et al. (2017) who have reported that endophytic strains associated with medicinal plants *Ziziphora capital* and *Ferula songorica* respectively, exhibited antifungal activity by producing several lytic enzymes.

To authenticate the results of *in vitro* studies, we selected endophytic isolates possessing multifarious PGPTs for *in vivo* experiments. In pot experiment, the application of endophytic strains significantly increased the physical growth parameters of *R. officinalis* seedlings over untreated control, especially *Pseudomonas aeruginosa* SI12, *Cedecea lapagei* KU14 and *Bacillus subtilis* KU21. The increased shoot/ root parameters in the inoculated plants is attributed to the release of a variety of plant growth regulators in the rhizosphere, resulting in an altered root architecture that may have prompted an expansion in the total root surface area and consequently, improved the water and nutrient uptake, especially N and P, with positive effects on plant growth as a whole (Montano et al. 2014). Similar results were documented by Gupta et al. (2016) with the isolates LS.B11 (*Pseudomonas* sp.) and EF.B3 (*Burkholderia* sp.) isolated from medicinal plants *Echinacea purpurea* and *Lonicera japonica* which showed an increase in various physical growth parameters of pea seedlings.

The inoculation of *R. officinalis* seedlings with indigenous endophytes was also reported to have affected several physiological properties of plants. The significantly increased contents of total chlorophyll were observed in plants inoculated with *Bacillis subtilis* KU21, followed by *Pseudomonas aeruginosa* SI12, *Cedecea lapagei* KU14. The results of the present study corresponded with those of Zhang et al. (2008), who reported that PGPR *B. subtilis* GB03 improved photosynthetic capability by augmenting photosynthetic efficiency and chlorophyll levels in *Arabidopsis*. Plants possess a variety of antioxidant molecules predominantly phenols that alleviate the reactive oxygen species and defend host cells against adverse conditions. The results of the present study suggested that there were certain elicitors in the microbial cultures which played a vital role in enhancing the phenolic content of *R. officinalis* leaves. Several studies have likewise reported the promising effect of endophytic bacteria in boosting phenolics and flavanoid contents in *Withania somnifera* (L.), Dunal, and sweet basil (Gupta and Pandey 2015; Singh et al. 2016).

In case of nutrient acquisition, an improved NPK concentration in plants inoculated with *Bacillis subtilis* KU21 followed by *Pseudomonas aeruginosa* SI12, *Cedecea lapagei* KU14 was observed. This enhanced capacity of the plant to attain and utilize more nutrients could be attributed to the bioinoculation effect on the stimulated root system (Egamberdieva et al. 2017). Moreover, these microbes are also capable of solubilizing mineral nutrients, resulting in increased levels of available NPK in the soil, thereby facilitating their availability to plants (Setiawati and Mutmainnah 2016). For example, *Bacillus* and *Pseudomonas*, possessing mineral solubilizing and nitrogen-fixing ability, significantly increased NP uptake in *Zea mays* L (Zahid et al. 2015). The endophytic strains of the current investigation were found to be capable of solubilizing P and fixing N under *in vitro* conditions, thus providing more NP to *R. officinalis* seedlings.

In a nutshell, among the eleven selected bacterial endophytes for plant growth promotion experiment, *B. subtilis* KU21, *P. aeruginosa* SI12, and *C. lapagei* KU14 improved the growth parameters of *R. officinalis* significantly. Moreover, these isolates were also resistant to various combinations of antibiotics. The PGP bacteria resistant to antibiotics may have survival and competitive qualities required for a good bioinoculant to be used as biofertilizer (Kloepper et al. 1980). Further field trials for exploring the future application of these strains in enhancing *R. officinalis* productivity are well under way. The current study has also reported the first time occurrence of *C. lapagei* as an endophytic bacterium from *R. officinalis*. Although, some studies have reported *Cedecea lapagei* as an inhabitant of rhizo and endorhizosphere possessing multiple plant growth-promoting traits (Zhang et al. 2015; Benaissa et al. 2019). The *in vivo* growth promotion studies for this strain have not been evaluated so far. The current findings point out the first ever evidence of the growth promoting potential of *C. lapagei* on *R. officinalis* under *in vivo* conditions.

Declarations

Acknowledgments

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Conflict of interest

The authors have declared no conflict of interest.

References

Abbamondi GR, Tommonaro G, Weyens N, Thijs S, Sillen W, Gkorezis, P (2016) Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. Chem Biol Technol Agric 3. http://doi.org/10.1186/s40538-015-0051-3

Abdelshafy Mohamad OA, Ma JB, Liu YH, Zhang, D, Hua S, Bhute S (2020) Beneficialendophytic bacterial populations associated with medicinal plant *Thymus vulgaris* alleviate salt stress and confer resistance to *Fusarium oxysporum*. Front Plant Sci 11:1–17.

Altschul SF, Thomas LM, Alejandro AS, Jinghui Z, Zheng Z, Webb M, David JL (1997) Gapped BLAST and PSIBLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389-3402.

Bacon CW, White JF (2016) Functions, mechanisms and regulation of endophytic and epiphytic microbial communities of plants. Symbiosis 68: 87–98.

Baker AW, Schippers S (1987) Microbial cyanide production in the rhizosphere about potato yield reduction and *Pseudomonas* sp. mediated plant growth stimulation. Soil Biol Biochem 19: 451-457.

Benaissa A, Djebbar R, Abderrahmani A (2019) Diversity of plant growth-promoting rhizobacteria of *Rhus tripartitus* in the arid soil of Algeria (Ahaggar) and their physiological properties under abiotic stresses. Adv Hortic Sci 32: 525-534.

Bourhia M, Laasri FE, Aourik H, Boukhris A, Ullah R, Bari A, Ali SS, Mzibri El M, Benbacer L, Gmouh S (2019) Antioxidant and Antiproliferative Activities of bioactive compounds contained in *Rosmarinus officinalis* Used in the Mediterranean Diet. Evid.-based complementary altern. Med. https://doi.org/10.1155/2019/7623830

Brezeanu C, Variaţia PF (2005) Permeability membrane or şi conţinutul de clorofila din frunzele de pepene galben in timpul procesului de crestere. Lucrari Stiintifice Seria Horticultura 48: 635-638.

Cappuccino JC, Sherman N (1992) Microbiology: "A Laboratory Manual." 3rd ed. Benjamin/Cummings, Pub. 125-179.

Cordero I, Ruiz-Díez B, Balaguer L, Richter A, Pueyo JJ, Rincon A (2017) Rhizospheric microbial community of *Caesalpinia spinosa* (Mol.) Kuntze in conserved and deforested zones of the Atiquipa fog forest in Peru. Appl Soil Ecol 114: 132–141.

de Favaro LCL, de Sebastianes FLS, Araujo WL (2012) *Epicoccum nigrum* P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PLoS One. http://doi.org/10.1371/journal.pone.0036826

Dehghani Bidgoli R, Azarnezhad N, Akhbari M, Ghorbani M (2019) Salinity stress and PGPR effects on essential oil changes in *Rosmarinus officinalis* L. Agric Food Secur 8: 1–7.

Egamberdieva D, Wirth S, Behrendt U, Ahmad P, Berg G (2017) The antimicrobial activity of medicinal plants correlates with the proportion of antagonistic endophytes. Front Microbiol 8: 199p.

Elmagzob AAH, Ibrahim MM, Zhang GF (2019) Seasonal diversity of endophytic bacteria associated with *Cinnamomum camphora* (L.) Presl. Diversity. http://doi.org/10.3390/d11070112

Faust SD, Mikulewicz EW (1967) Factors influencing the condensation of 4 aminoantipyrine with derivatives of hydroxybenzene – II. Influence of hydronium ion concentration on absorptivity. Water Res 1: 509–522.

Fleming HP, Etchells JL, Costilus RH (1975) Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. J Appl Microbiol 30: 1040-1042.

Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: Bacterial root endophytes and plant growth promotion. Am J Bot 100: 1738–1750.

Glick BR (2012) Plant growth-promoting rhizobacteria: Mechanisms and Applications. Scientificia. http://doi.org/ 10.6064/2012/963401

Gordon SA, Palleg LG (1957) Quantitative measurement of IAA .Plant Physiology 10:37-38.

Gupta H, Saini RV, Pagadala V, Kumar N, Sharma DK, Saini AK (2016) Analysis of plant growth-promoting potential of endophytes isolated from *Echinacea purpurea* and *Lonicera japonica.* J Soil Sci Plant Nutr 16: 558-577.

Gupta R, Pandey R (2015) Microbial interference ameliorates essential oil yield and diminishes root-knot infestation in sweet basil under field conditions. Biocontrol Sci Technol 25: 1165-1179.

Helrich K (1990) Official and tentative methods of analysis. Association of Official Analytical Chemists. Washington:William Star Wetglad.

Higgins DJ, Thompson T, Gibson JD, Thompson DGH, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res 22: 4673-4680.

Holt JG, Krieg NR, Sneath PHA, Staley JT (1994) Bergey's manual of Determinative Bacteriology, 9th edn. Williams and Wilkins, Baltimore, (MD).

Jackson ML (1967) Soil chemical analysis. Bombay: Oxford and IBH Publishing House; p. 38.

Jackson ML (1973) Soil chemical analysis. New Delhi: Prentice-Hall of India.

Jensen ES (1987) Inoculation of pea by *Rhizobium* in planting furrow. Plant Soil 97: 63-70.

Khamwan S, Boonlue S, Riddech N, Jogloy S, Mongkolthanaruk W (2018) Characterization of endophytic bacteria and their response to plant growth promotion in *Helianthus tuberosus* L. Biocatal Agric Biotechnol 13: 153–159.

Kloepper JW, Schrot MN, Miller TD (1980) Effect of rhizosphere colonization by plant growth promoting rhizobacteria on potato development and yield. Phytopathology 70: 1080-1082.

Liotti, RG, da Silva Figueiredo MI, da Silva GF, de Mendonça EAF, Soares MA (2018) Diversity of cultivable bacterial endophytes in *Paullinia cupana* and their potential for plant growth promotion and phytopathogen control. Microbiol Res 207: 8–18.

Liu Y, Guo J, Li L, Asem MD, ZhangnY, Mohamad OA (2017) Endophytic bacteria associated with endangered plant *Ferula sinkiangensis* in an arid land: diversity and plant growth-promoting traits. J Arid Land 9: 432-445.

Maggini V, Miceli E, Fagorzi C, Maida I, Fondi M, Perrin E (2018) Antagonism and antibiotic resistance drive a species-specific plant microbiota differentiation in *Echinacea* spp. FEMS Microbiol Ecol. http:// doi.org/10.1093/femsec/fiy118

McMullin DR, Nguyen HDT, Daly GJ, Menard BS, Miller JD (2018) Detection of foliar endophytes and their metabolites in Picea and Pinus seedling needles. Fungal Ecol 31: 1–8.

Miller KI, Qing C, Sze DM, Neilan BA (2012) Investigation of the biosynthetic potential of endophytes in traditional Chinese anticancer herbs. PLoS ONE. http://doi.org/10.1371/journal.pone.0035953.

Mohamad OAA, Li L, Ma JB, Hatab S et al (2018) Evaluation of the antimicrobial activity of endophytic bacterial populations from chinese traditional medicinal plant Licorice and characterization of the bioactive secondary metabolites produced by *Bacillus atrophaeus* against *Verticillium dahlia.* Front Microbiol. https://doi.org/10.3389/fmicb.2018.00924.

Montano PF, Villeges AC, Bellogin RA et al (2014) Plant growth promotion in cereal and leguminous agricultural important plants: from microorganism capacities to crop production. Microbiol Res 169: 325–326.

Mortensen CN (1997) Seed bacteriology laboratory guide. Copenhagen (Denmark): Danish Government Institute of Seed Pathology for Developing Countries.

Passari AK, Mishra VK, Leo VV, Gupta VK, Singh BP (2016) Phytohormone production endowed with antagonistic potential and plant growth-promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. Microbiol Res 193: 57-73.

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Journal of Microbiologiya. 7: 362-370.

Purushotham N, Jones E, Monk J, Ridgway H (2020) Community Structure, Diversity and Potential of endophytic bacteria in the primitive New Zealand medicinal plant *Pseudowintera colorata*. Plants. http://doi.org/10.3390/plants9020156.

Robert WK, Selitrennikoff CP (1988) Plant and bacterial chitinase differ in antifungal activity. J Gen Microbiol 134: 169-176.

Sadegh Kasmaei L, Yasrebi J, Zarei M, Ronaghi A, Ghasemi R, Saharkhiz MJ (2019) Influence of plant growth promoting rhizobacteria, compost, and biochar of Azolla on Rosemary (*Rosmarinus Officinalis* L.) growth and some soil quality indicators in a calcareous soil. Commun Soil Sci Plant Anal 50: 119–131.

Sambrook J, Fritsch EF, Maniatis T (1989) Diversity, biocontrol, and plant growth-promoting abilities of xylem residing bacteria from solanaceous crops. Int J Microbiol Res 38: 14p.

Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183: 92–99.

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160: 47-56.

Setiawati C, Mutmainnah L (2016) Solubilization of potassium-containing mineral by microorganisms from the sugarcane rhizosphere. Agric Sci Procedia 9: 108-117.

Shaw FJ, Lin PF, Chen CS, Chen CH (1995) Purification and characterization of an extracellular α- amylase from thermos species. Bot Bull Acad Sin 36: 195-200.

Silva CF, Vitorino LC, Mendonça MAC, Araujo WL, Dourado MN, Albuquerque LC (2019) Screening of plant growth-promoting endophytic bacteria from the roots of the medicinal plant Aloe vera. South African J Bot. http:// doi.org/10.1016/j.sajb.2019.09.019

Singh A, Gupta R, Srivastava M, Gupta MM, Pandey R (2016) Microbial secondary 637 metabolites ameliorate growth, in planta contents and lignification in *Withania somnifera* (L.) 638 Dunal. Physiol Mol Biol Pla 22: 253-260.

Sun H, Kong L, Du H, Chai Z, Gao J, Cao Q (2019) Benefits of *Pseudomonas poae* s61 on *Astragalus mongholicus* growth and bioactive compound accumulation under drought stress. J Plant Interact 14: 205–212.

Tandon HLS (2009) Methods of analysis of soil, plant, water, fertilizers, and organic manures. (Fertiliser Development and Consultation Organisation: New Delhi).

Vincent JM (1947) Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 150: 850p.

Witham FH, Bladydes DF, Delvins RM (1971) Experiments in plant physiology. New York: Van Nostrand Reinhold; p. 245

Yuan B, Wang Z, Qin S, Zhao Gui-H, Feng, YJ, Wei Li-H, Jiang Ji-H (2012) Study of the anti-sapstain fungus activity of *Bacillus amyloliquefaciens* CGMCC 5569 associated with Ginkgo biloba and identification of its active components. Bioresour Technol 114: 536–541.

Zahid M, Abbasi MK, Hameed S, Rahim N (2015) Isolation and identification of indigenous plant growth-promoting rhizobacteria from the Himalayan region of Kashmir and their effect on improving growth and nutrient contents of maize (*Zea mays* L.). Front Microbiol 6: 207p.

Zhang H, Xie X, Kim MS, Kornyeyev DA, Holaday S, Pare PW (2008) Soil bacteria augment Arabidopsis photosynthesis by decreasing glucose sensing and abscisic acid levels *in planta*. Plant 6: 264-273.

Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y (2015) Screening and characterization of endophytic Bacillus and Paenibacillus strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz J Microbiol . http://doi.org/10.1590/S1517-838246420140024

Tables

Table 1 Beneficial plant traits of endophytic bacteria under *in vitro* conditions.

Endophytic strains	Biofertilizer activities				Antagonistic properties					Biocont
	Phosphate solubilization ($\mu g\ ml^{-1}$)	IAA production ($\mu g\ ml^{-1}$)	Nitrogen fixing ability*	Siderophore production (%SU)	Lytic enzymes**			HCN Production#	Ammonia production##	*F.oxysp*
					Chitinase	Protease	Amylase			
P. mediterranea KA7	170.00 ±2.88^{fghi}	35.00± 1.73^{f}	++	74.32 ±1.16^{gh}	+	-	++	+	+	76.66 (61.10)^{b}
P. oryzihabitans KU5	200.00 ±1.15^{e}	65.00± 2.88^{a}	++	48.56 ±0.72^{j}	++	+	++	++	++	55.55 (48.16)^{c}
B. flexux KA10	145.00 ±4.04^{j}	15.00± 0.58^{j}	++	78.23± 1.59^{fg}	-	+++	+	++	+	0.00
P. aeruginosa SI12	176.00 ±1.73^{fg}	48.00 ±2.89^{bcd}	+++	102.34±2.06^{de}	++	++	+++	+++	+++	77.77 (61.83)^{a}
P. japonensis KU13	175.00 ±2.88^{fgh}	28.00 ±1.73^{gh}	+++	166.67± 2.43^{c}	++	++	++	++	++	54.76 (47.72)^{c}
P. putida KU2	215.00± 5.77^{d}	24.00 ±1.73^{ghi}	++	220.31± 0.78^{b}	+++	+++	+	++	++	44.44 (41.78)^{f}
C. lapagei KU14	250.00 ±2.31^{c}	30.00 ±2.89^{fg}	++	35.71± 2.45^{k}		++	+	+++	++	77.77 (61.90)^{a}
P. agglomerans KA14	320.00 ±6.92^{b}	30.00 ±1.73^{fg}	++	103.23 ±1.29^{d}	+++	+++	-	+++	+	76.66 (61.14)^{b}
P. koreensis KA11	180.00 ±2.31^{f}	49.00 ±3.46^{bc}	+	81.90±1.87^{f}	++	+	-	++	+	77.77 (61.90)^{a}
B. simplex KA2	115.00 ±1.15^{k}	42.00 ±1.16^{de}	+	71.43±2.19^{hi}	++	++	+	++	++	55.55 (48.17)^{c}
B. subtilis KU21	375.00 ±4.04^{a}	52.00± 2.31^{b}	+++	301.48±1.62^{a}	+++	++	+++	+++	++	60.00 (50.75)^{c}
LSD ($P \leq 0.05$)	**10.79**	**6.66**	**-**	**5.17**	**-**	**-**	**-**	**-**	**-**	**3.21**

*Values ranging from <3 mm (+) colony diameter; 3–6 mm (++) colony diameter; >6 mm (+++) colony diameter; **Values ranging from zone diameter of <1.5mm (+); 1.5–3mm (++); >3mm (+++); no activity (-) ; #light brown color (+); dark brown colour (++); orange brown colour (+++); no activity (-); ##(+) fair; (++) good; (+++) very good; ammonia producers; no activity (-); Values in parentheses are arc sine-transformed; With in a column, means followed by the same letter are not significantly different based on $LSD_{0.05}$.

Each value within columns represents mean of three replicates

Table 2 Effect of inoculation with endophytic bacteria on physical growth parameters of *R. officinalis*.

Treatment	Number of lateral branches	Stem height (cm)	Root length (cm)	Root biomass (g plant^{-1})	Shoot biomass (g plant^{-1})
T1 (Control)	12.33± 0.19^{jkl}	29.80 ±0.46^{l}	13.20± 0.12^{kl}	0.58± 0.05^{ijk}	3.35± 0.20^{hijkl}
T2 (*P. mediterranea* KA7)	15.88± 0.51^{de}	35.53± 0.71^{efg}	15.84± 0.49^{defg}	0.74± 0.02^{efgh}	3.90 ±0.52^{cdefgh}
T3 (*P. oryzihabitans* KU5)	15.53± 0.31^{defg}	36.11± 0.49^{def}	15.96 ±0.55^{def}	0.76± 0.03^{defg}	3.88 ±0.58^{cdefghi}
T4 (*B. flexux* KA10)	13.41± 0.62^{ij}	31.48± 0.66^{k}	14.89± 0.28^{efghij}	0.69± 0.04^{ghijk}	3.59 ±0.29^{fghijk}
T5 (*P. aeruginosa* SI12)	17.74± 0.43^{abc}	41.21± 0.43^{ab}	18.72± 0.42^{a}	0.99± 0.05^{a}	4.51 ±0.59^{abc}
T6 (*P. japonensis* KU13)	12.55 ±0.56^{jk}	33.51± 0.63^{j}	14.01± 0.58^{jk}	0.72± 0.03^{ghi}	3.69 ±0.17^{fghij}
T7 (*P. putida* KU2)	14.52 ±0.30^{ghi}	36.77± 1.01^{de}	16.08± 0.05^{de}	0.87± 0.04^{abcdef}	4.37 ±0.21^{abcd}
T8 (*C. lapagei* KU14)	17.95± 0.55^{ab}	38.45±0.91^{c}	17.46± 0.26^{bc}	0.94± 0.02^{ab}	4.59 ±0.42^{ab}
T9 (*P. agglomerans* KA14)	15.82± 0.47^{def}	35.13± 0.97^{ghi}	15.30± 0.17^{efgh}	0.90± 0.12^{abcd}	4.08 ±0.05^{bcdefg}
T10 (*P. koreensis* KA11)	14.95± 0.54^{efgh}	35.46± 1.19^{efgh}	15.23± 0.49^{efghi}	0.88± 0.06^{abcde}	4.09 ±0.21^{abcdef}
T11 (*B. simplex* KA2)	16.51 ±0.29^{cd}	37.51± 0.79^{cd}	16.61± 0.35^{cd}	0.92± 0.01^{abc}	4.35 ±0.20^{abcde}
T12 (*B. subtilis* KU21)	18.05± 0.58^{a}	41.29± 0.745^{a}	18.10± 0.58^{ab}	0.94± 0.03^{ab}	4.72 ±0.58^{a}
LSD ($P \leq 0.05$)	**1.27**	**1.49**	**1.19**	**0.14**	**0.63**

Values within column marked by same letters indicate significant differences based on least significant difference at $P \leq 0.05$.

Each value within columns represents mean of three replicates.

Table 3 Effect of inoculation with endophytic bacteria on soil characteristics (post trial).

Treatment	Soil chemical characteristics (Kg ha^{-1})			Total viable endophytic bacterial population (10^2×cfu g^{-1} root)	Population of inoculated endophytic bacteria (10^2×cfu g^{-1} root)	Simpson's index of Dominance (D)
	Available N	Available P	Available K			
T1 (Control)	298.47± 0.58^{l}	25.90± 0.52^{kl}	321.09± 0.57^{kl}	31.00± 1.16^{ij}	0.00	0.00
T2 (*P. mediterranea* KA7)	319.21± 1.15^{i}	27.52± 0.3^{fghijk}	327.62± 1.15^{efg}	49.00± 4.04^{bc}	35.00± 1.15^{cd}	0.51± 0.06^{cdefgh}
T3 (*P. oryzihabitans* KU5)	326.09± 0.04^{gh}	27.73± 1.15^{fghi}	328.04± 1.62^{ef}	41.00± 2.31^{ef}	32.00± 1.21^{def}	0.61± 0.04^{abcd}
T4 (*B. flexux* KA10)	305.01± 1.23^{jk}	27.83± 1.15^{fgh}	322.17± 0.08^{ijk}	35.00± 1.73^{hi}	25.00± 2.31^{hi}	0.51± 0.07^{cdefgh}
T5 (*P. aeruginosa* SI12)	334.27± 0.06^{c}	30.31± 2.02^{abc}	326.24± 0.58^{fgh}	51.00± 1.16^{ab}	43.00± 1.73^{b}	0.71± 0.05^{ab}
T6 (*P. japonensis* KU13)	308.64± 2.13^{j}	27.55± 0.58^{fghij}	324.82± 0.54^{ghi}	37.00± 3.46^{fgh}	26.00± 0.58^{h}	0.49± 0.06^{cdefghi}
T7 (*P. putida* KU2)	328.38± 0.44^{defg}	30.18± 0.11^{bcd}	324.31± 1.10^{ij}	43.00± 1.73^{de}	33.00± 0.57^{de}	0.59± 0.10^{bcdef}
T8 (*C. lapagei* KU14)	338.98± 1.16^{ab}	31.42± 0.52^{ab}	333.21± 0.14^{cd}	49.00± 2.88^{bc}	38.00± 1.73^{c}	0.60± 0.46^{bcde}
T9 (*P. agglomerans* KA14)	331.60± 0.58^{cde}	29.54± 0.31^{cde}	330.72± 1.48^{de}	43.00± 1.15^{de}	32.00± 0.58^{def}	0.55± 0.07^{bcdefg}
T10 (*P. koreensis* KA11)	331.05± 1.73^{cdef}	28.43± 0.25^{efg}	335.54± 0.32^{bc}	39.00± 2.02^{efg}	31.00± 1.73^{efg}	0.63± 0.06^{abc}
T11 (*B. simplex* KA2)	332.15± 0.58^{cd}	28.59± 1.04^{ef}	338.07± 0.58^{ab}	47.00± 3.46^{bcd}	35.00± 2.31^{cd}	0.55± 0.05^{bcdefg}
T12 (*B. subtilis* KU21)	339.06± 1.61^{a}	31.88± 0.51^{a}	339.29± 1.73^{a}	54.00± 2.31^{a}	48.00± 2.89^{a}	0.79± 0.07^{a}
LSD ($P \leq 0.05$)	4.09	1.58	3.19	4.24	3.77	0.18

Within columns, means followed by same letter are not significantly different (least significant difference (LSD) at P ≤ 0.05).

Each value within columns represents mean of three replicates.

Table 4 Antibiotic sensitivity test of endophytic bacteria

Antibiotics	Concentration (µg disc^{-1})	Endophytic strains		
		B. subtilis KU21	*P. aeruginosa* S112	*C. lapagei* KU14
Hexa universal-1				
Bacitracin	10	-	-	-
Chloramphenicol	30	-	-	-
Penicillin G	10	-	-	-
Polymyxin B	300	-	+	-
Gentamicin	10	-	-	-
Neomycin	30	-	-	+
Hexa universal -2				
Cefotaxime	30	-	-	-
Augmentin	30	-	-	-
Erythromycin	10	-	-	-
Chloramphenicol	30	-	-	-
Ofloxacin	5	-	-	-
Co-trimoxazole	25	-	-	+
Hexa Pseudo 1				
Cefoperazone	75	-	-	-
Piperacillin	100	-	-	-
Levofloxacin	5	-	-	-
Gentamicin	10	-	-	-
Amikacin	30	+	+	+
Colistin	10	-	+	-
Hexa Pseudo 2				
Imipenem	10	-	-	-
Aztreonam	30	-	-	-
Sulbactam	10	-	-	-
Tazobactam	10	-	-	-
Ceftazidime	30	-	-	-
Netillin	30	-	-	-

- = Resistant; + = Susceptible

Figures

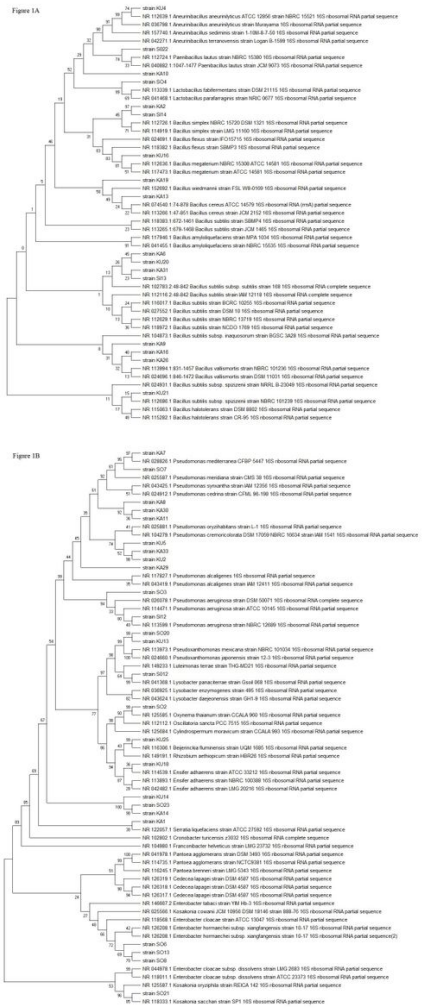


Figure 1

Phylogenetic tree based on 16S rDNA sequences of gram positive bacteria showing the relationship between endophytes sequences closest type strain sequences (A); Phylogenetic tree based on 16S rDNA sequences of gram negative bacteria showing the relationship between endophytes sequences closest type strain sequences (B).

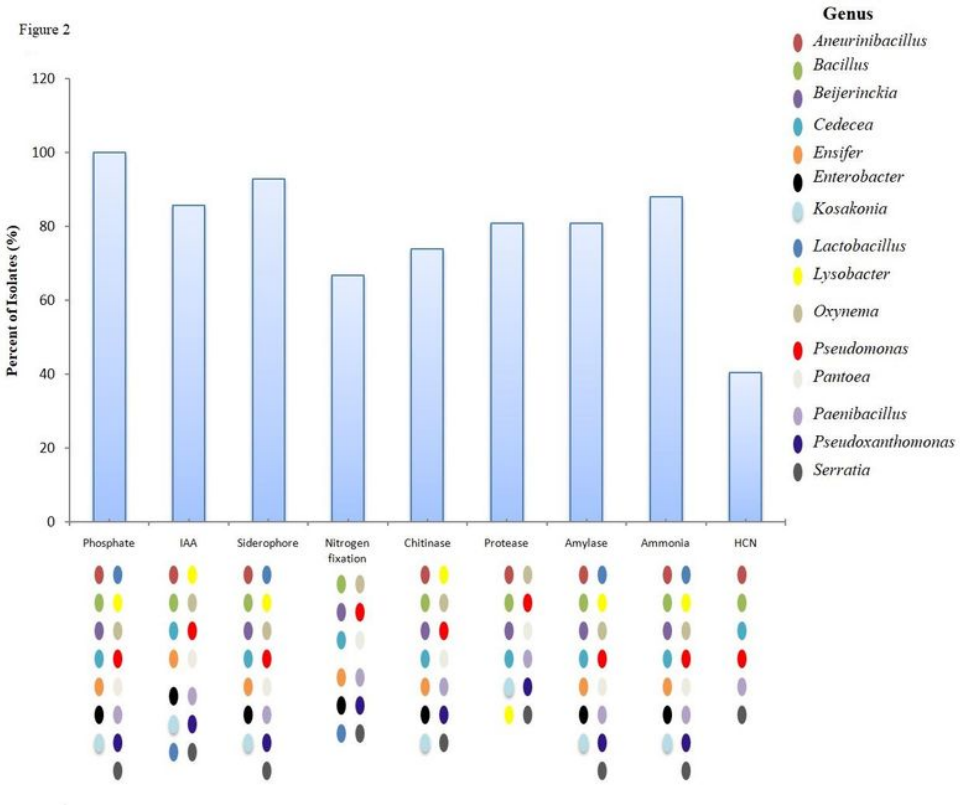


Figure 2

In vitro screening for beneficial plant traits of bacterial strains. Colored dots represent different genus in which activities were observed.

Figure 3

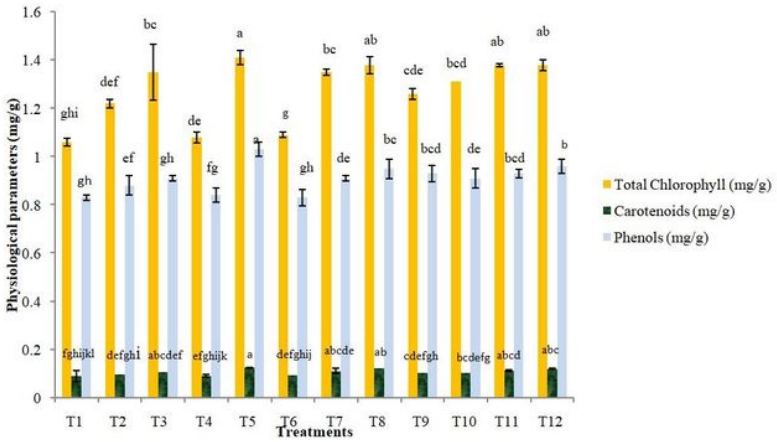
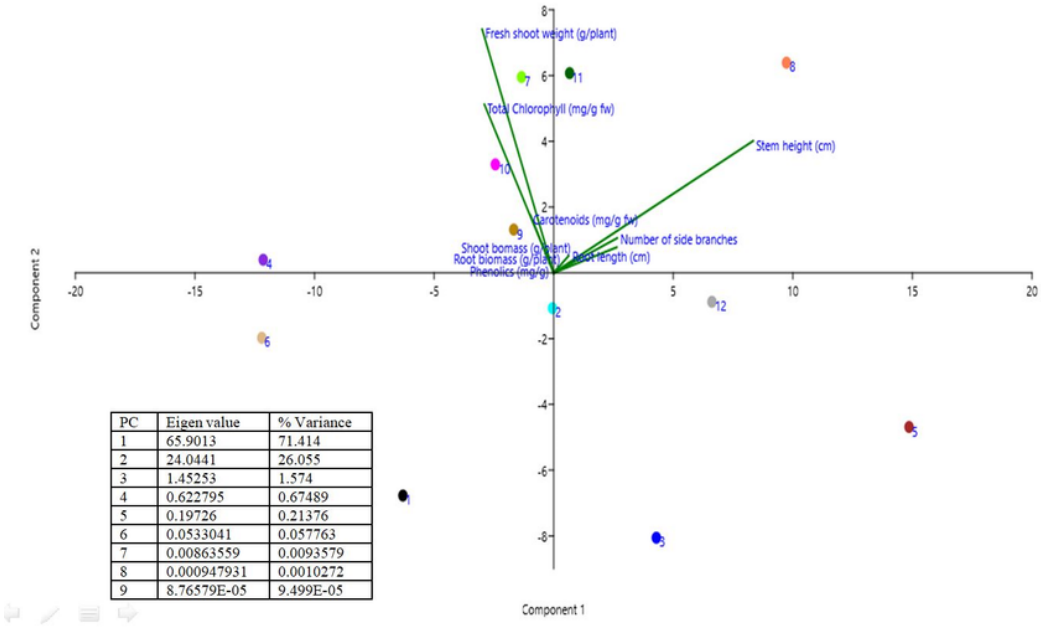


Figure 3

Effect of inoculation with endophytic bacteria on physiological characteristics of R. officinalis. Bars marked by different letters indicate significant differences based on LSD at P≤0.05; error bars indicate means ± SE by LSD test (n = 3). T1 (Control); T2 (P. mediterranea KA7); T3 (P. oryzihabitans KU5); T4 (B. flexux KA10); T5 (P. aeruginosa SI12); T6 (P. japonensis KU13); T7 (P. putida KU2); T8 (C. lapagei KU14); T9 (P. agglomerans KA14); T10 (P. koreensis KA11); T11 (B. simplex KA2); T12 (B. subtilis KU21).

Figure 4



PC	Eigen value	% Variance
1	65.9013	71.414
2	24.0441	26.055
3	1.45253	1.574
4	0.622795	0.67489
5	0.19726	0.21376
6	0.0533041	0.057763
7	0.00863559	0.0093579
8	0.000947931	0.0010272
9	8.76579E-05	9.499E-05

Figure 4

PCA of growth characteristics of R. officinalis in response to endophytic bacterial isolates inoculation.1-T1 (Control), 2-T2 (P. mediterranea KA7), 3-T3(P. oryzihabitans KU5), 4-T4 (B. flexux KA10), 5-T5 (P. aeruginosa SI12), 6-T6 (P. japonensis KU13), 7-T7 (P. putida KU2), 8-T8 (C. lapagei KU14), 9-T9 (P. agglomerans KA14), 10-T10 (P. koreensis KA11), 11-T11 (B.simplex KA2), 12-T12 (B.subtilis KU21).

Figure 5

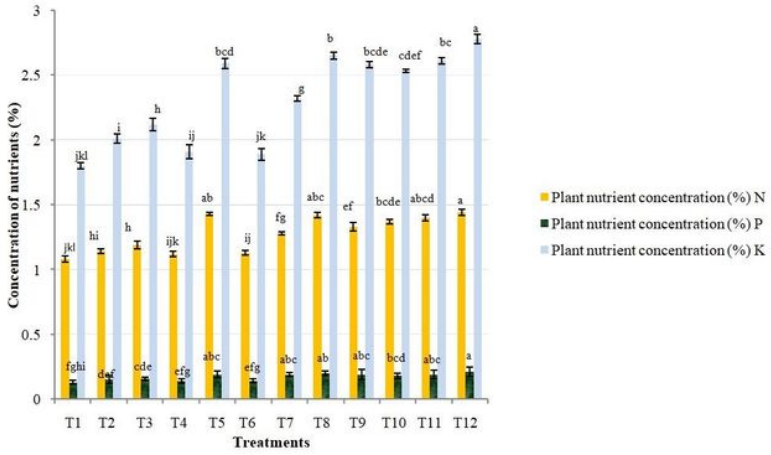


Figure 5

Effect of liquid bacterial inoculum on nutrient concentration of R. officinalis. Bars marked by different letters indicate significant differences based on LSD at P≤0.05; error bars indicate means ± SE by LSD test (n = 3). T1 (Control); T2 (P. mediterranea KA7); T3 (P. oryzihabitans KU5); T4 (B. flexux KA10); T5 (P. aeruginosa SI12); T6 (P. japonensis KU13); T7 (P. putida KU2); T8 (C. lapagei KU14); T9 (P. agglomerans KA14); T10 (P. koreensis KA11); T11 (B.simplex KA2); T12 (B. subtilis KU21).

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