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Microbial symbiosis and microbiological synergy with oak trees

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Abstract

The oak trees are one of the typical crucial ecosystems placed in western Iran situated in Kermanshah province. Due to human manipulation, ecological and climate change are deteriorating. In this investigation, microbial isolation and treatments were offered to evaluate their effects on *oak* growth.

Samples collected from *Quercus* brant ii of Kermanshah regions, Iran, and were cultivated on ISP2 and NA medium, kept at 28 °C for one month. The Microscopic identification and phosphate solubilization assay for primary and nitrogen fixation, hydrogen cyanide, protease, and phytohormone production performed. Lastly, Seedlings with selected isolate treated and growth and survival parameters measured.

All strains were able to produce auxin and gibberellin in different values. All of the isolates able to solubilize phosphate. The outcomes of nitrogen fixation ability, protease, and siderophore production diverged among strains. Picked isolates exhibited hormone production properties as well as biocontrol influences according to tests. Co-treatment of *Streptomyces* sp. Qb2-*Pseudomonas* sp. Qb, *Streptomyces* sp. Qb2-*Bacillus* sp. Qb and *Streptomyces* sp. Qb2- *Pseudomonas* sp. Qb had a notable effect on seedling growth parameters (P <0 ·001), *oak* species survival (P <0 ·001), and improved significantly the longitudinal growth, diameter, and dry weight percent of seedlings (P <0 ·005) Respectively. *Streptomyces* sp. Qb2 and *Bacillus* sp. Qb strains had the highest and lowest inhibition effects on bacterial plant pathogen, *Pseudomonas* syringae. *Streptomyces* sp. Qb2 strains significantly promote root formation. To our understanding, this is the first record of *Streptomyces* endophyte isolation from Iranian *oak* trees with biocontrol and growth-inducing impacts. It is also proposed to apply the co-treatment plan to increase biocontrol effects and growth induction.

Keywords: Oak, **symbionts** bacteria, co-treatment, *Streptomyces*, *Quercus* brant ii.

Introduction

The oak forests are one of the typical crucial ecosystems placed in western Iran. A remarkable area of these forests situated in Kermanshah province. The foremost tree species in the area is *Quercus* brant ii (Qb), with 70% of the Zagros forest species being *oak* [1]*Quercus* is one of the most recognized genera of *Fagaceae* in courses of species, geographic distribution, environmental rank, and ecological, and economic state. Due to community growth, fuel consumption, livestock feed, land expansion, road building, electricity, and telephone lines, planned development, industrial projects, and plant pathogens, these forests are on the deterioration[2]. Across the last three decades, the presence of endophytic symbiont microorganisms has been shown to serve their host[3]. Endophytic symbiont bacteria improve plant growth, ability to solubilize phosphate, and contribute assailable nitrogen to plants. They have active cooperation[4] or that these symbiotic microorganisms enhance the host's ability to adapt and survive and to grow.

The name endophyte introduces the permanence of microorganisms inside the crowded plant tissues outdoors, having adverse impacts on the organism plant [5] The bacteria are farmers of growth-promoting metabolites, insect and germ repellents, antimicrobials facing plant pathogens, supporters in pressure states, and multiple more extended [6] They additionally maintain the potential to deliver novel auxiliary metabolites, which can employ in pharmaceutical, agricultural, and other manufacturers.

These bacteria colonize every similar environmental niche in plants as plant pathogens. They have extensively recognized tools of biocontrol motion before-mentioned as they fight for ecological niche or substrate, making of inhibitory compounds, and induced systemic confidence in the host toward a full spectrum of pathogens[7]. Also, the synergies between plants and entophytic bacteria might help plants to settle in ecosystem recovery methods. Individual plant species might be a host for one or more further entophytic bacterial species. The presence of entophytic bacterial species and their population density is genuinely variable depending on the bacterial varieties, hostess genotypes, and environmental states[8]. Thus, there is a developing interest in bioprospecting of endophytic symbiont microbial communities from various ecosystems. Among which *actinobacteria* required because of the production of different biologically active materials of commercial value. Rhizobacteria, bacteria, epiphytes, and endophytes are also important. The role of colonizing bacteria in plant tissues has studied[9]. Endophytes can affect germination, plant propagation under different conditions, promote plant growth, or limit infection of

plant pathogens [10]. Other gains of entophytic symbiont bacteria such as nitrogen fixation, immune system inducers and enhancers of available minerals and reduced ethylene production lead to survival and adaptation of the host[11]. It also promotes disease resistance and adaptive power by producing a wide range of secondary metabolites[12] and stimulates plant host growth.

The inherent symbiotic bacteria isolated from forest trees

include *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Actinobacteria*, *Sphingomonas*, and *Enterobacteriaceae* [13]. Investigations in the area of endophyte *actinomycetes* from different environments are insufficient. The only study in Iran focuses on gram-negative endophytes. (Marivan) Forests have investigated[14]. Nevertheless, the search for gram-positive *actinomycetes* has not conducted. Furthermore, only the study of *actinomycetes* affecting physiological and pathological processes[15]has investigated.

The management, restoration, and regeneration of forests using the biological capabilities of active microbial metabolites have so far been neglected in Iran, notably in the western Zagros and Kermanshah provinces, to control the deterioration of *oak* trees. Two areas, which have very positive economic and environmental potential, are biocontrol and biodegradation, research. Which, if the objectives of this study succeed, choose the isolates as biocontrol and biodegradation. This study aimed to evaluate the effects of oak endophytes on growth parameters and survival.

Material and methods

locations

Zagros are oak forests that dominate between 1000 and 2000 m elevation and account for nearly 40% of Iran's covers[16]. This study was carried out in five natural forest stands dominated by Persian *oak* (*Qb*.) located in the west of Iran, including: Dalahoo 1 (34° 23' 28 N, 46° 03' 20 E), Kerend (34° 20' 19 N, 46° 07' 42 E), Dalahoo2 (34° 10' 42 N, 45° 22' 15 E), Islam Abad (34° 10' 43 N, 46° 39' 01 E).

2.2 Sampling

Rhizosphere soil of *oak* trees (Q. brant ii) sampled at different stations in the spring, and 50 g of samples transferred to the laboratory (4 °C). Homogeneous soil samples counted until the experiment. Plant elements such as leaves, stem, roots prepared freshly or saved at 4 °C until isolation in 24 h.

Cultivation of bacteria

Samples treated initially; the examples rinsed with distilled water. Leaf sample surfaces decontaminated

with 5% sodium hypochlorite for 5 min and three times. 50 µl of the final wash was cultured NA[1]medium to assay sterilization. The tissue sections were then cut and suspended in 5ml distilled water for 30 min and dried on autoclaved paper filters[17] .These explants cleaned in running tap water to murder adhered epiphytes, soil particles on the outside. Then sterilization by ethanol for 2–4 min performed[18]. Treatment methods including((NaClO3) 5% (Na2S2O3), 2.5%, ethanol 75% and (NaHCO3) 10%) were used to disinfect and repress fungal extension. The force of cleaning chemicals depends on the permeability of the sample. Contrarily, the inside tissues disinfect[19] . All the explants were lastly washed with pure distilled water, divided into small scraps (for steam or roots 1 cm and leaves1 cm2) and suspension injected on suitable agar medium.

After pre-treatment, 10 g of the soil sample was well homogenized, dried, and mixed with saline, and the Dilution serial was prepared (10-1 to 10-5). A loop of the desired dilution of each sample was cultured on the ISP2 medium[2] (g L−1): (Yeast extract (Difco)4.0 g, Malt extract (Difco) 10.0 g, Dextrose (Difco) 4.0 g, Agar 20.0 g 1000.0 ml Distilled water pH 7.2). The media were enriched using nystatin (50 µg /ml) to suppress fungal growth and stored at 30 °C for one month. Single colonies with unique *actinomycete* morphology arising out from the plant mass are separate. The purified cultures of the isolates collected by streaking on fresh media plates. Colonies with different appearance and sporulation determined for subsequent stages and microscopic and biochemical identification. *Actinomycetes* isolated by plating serial dilutions on the ISP2 medium. After one h of incubation at 90 °C, purified cultures were taken from picked colonies for renewed sub culturing on casein agar. The appearance characteristics were identified based on the color of the spore chain, surface mycelium color, soluble pigment color, and spore morphology[15]. Then, for the identification of strains gram and biochemical tests done.

Phosphate dissolution, Hormonal Assessment (Indole acetic acid (IAA) and gibberellic acid (GA) production)

The ability of the isolates to dissolve phosphate was calculated. Petri dishes containing the Picosawa[3] The culture medium was treated with 2 µl of suspension and incubated at 28 °C for seven days. The

[1] Nutrient agar
[2] International *Streptomyces* Project 2 medium
[3] (g l−1: glucose10; Ca3(PO4)2, 5; (NH4)2SO4, 0.5; NaCl, 0.2; MgSO4.7H2O, 0.1; KCl, 0.2; yeast extract, 0.5; MnSO4.2H2O, 0.002; and FeSO4.7H2O, 0.002.

generation of transparent halo near the colony indicates the dissolution of phosphate[20]. Screening of isolates for MPS[4] the activity performed on (HAP)[5] At 5 g /L as a single phosphate source[21]. Following seven days of incubation at 30°C, the plates examined for the presence of colonies developing apparent haloes (zone of solubilization). Spores of the *actinomycetes* isolates showing the largest halo zones were stored in 20% (w/v) sterile glycerol at −80°C until further use.

For this bacterial assay, isolates were cultured in 25 ml broth medium with 2% L-tryptophan and incubated at 28 °C for one week. Then The media centrifuged for 10 min (2100 rpm), and 1 ml of supernatant added to a 2 ml tube containing Salkowski's solution. The resulting mixture incubated in the dark for 45 min. The red color indicates the production of the endothelial hormone. A spectrophotometer read the adsorbed combination at 530 nm. The concentration of indoleacetic acid produced by the isolates was calculated using the curve plotted by standard indole acetic acid (Merck, Frankfurt, Germany) at dilutions of 0, 3, 6, 12, 25, 50, 125 and 250 µg /ml (Rahman, Sitepu, et al. 2010). In another measurement, the Gibberellic production rate was calculated using the Holbrook method[22]. In summary, the 24-hour culture of the bacteria was inoculated in Jenson broth medium[6]Furthermore, incubated at 200 °C for 24 hours. After centrifugation at 1850 for 2 min, the resulting supernatant was filtered and acidified with second hydrochloric acid to pH = 1-2 and concentrated and purified by ethyl acetate volume three times. The resulting gibberellic acid was separated again by the gradual addition of 15.20 and 10 ml of phosphate buffer at pH = 7.2. Each extract was added to a 100 ml flask and read at 254 nm. Gibberellic acid concentration of each isolate was determined using Gibberellic acid standard (Merck, Frankfurt, Germany) at levels of 0, 3, 6, 12, 25, 50, 125, and 250 µg /ml and curve plotting.

Plant growth-promoting potential of endophytic symbiont bacteria

Nitrogen fixation, Protease and hydrogen cyanide Production

Nitrogen-free semisolid malate[7] Medium made with or without ammonium chloride as a nitrogen source.

[4] Mineral phosphate-solubilizing
[5] Hydroxyapatite
[6] Ingredients g L-1: Sucrose 20.000, Dipotassium phosphate 1.000, Magnesium sulfate 0.500, Sodium chloride 0.500, Ferrous sulphate 0.100, Sodium molybdate 0.005 Calcium carbonate 2.000.

[7] DL-malic acid: 5.0 g, K_2HPO_4: 0.5 g, $MgSO_4 \cdot 7H_2O$: 0.2 g, NaCl: 0.1 g, $CaCl_2 \cdot 2H_2O$: 0.02 g. Micronutrient solution: 2 ml, Bromthymol blue solution (0.5% in 0.2N KOH): 2 ml, Fe(III) EDTA (1.64%): 4.0 ml, Vitamin solution: 1.0 ml, Distilled water: 1.0 L, Adjust pH to 6.8, For semisolid medium, add 0.5 g of agar; for solid medium, add 15 g of agar. Autoclave at 121°C for 15 min. Micronutrient solution: $CuSO_4 \cdot 5H_2O$:

After sterilization with an autoclave for 15 min, pre-plating the vitamin solution containing biotin and peroxide added. The isolates were inoculated and incubated for seven days at 28 °C to observe incubation growth[23] and[24].

Protease production performed according to the method of Sgroy, Petri dishes inoculated with 10 µl of bacterial isolates in Schim Milk Agar[8] Medium and incubated at 28 °C for 4 days. The formation of a bright halo around the bacterial colonies indicates the presence of an alkaline protease enzyme[24].

All isolates were screened to produce hydrogen cyanide by the method of Alstrom and Burns (1989)[25]. Fifty microliters of each bacterial suspension were cultured on the NA medium containing Whitman paper impregnated with the picric acid solution inside the plate. The culture plates were blocked with parafilm and incubated at 28-28 °C for seven days. The change of yellow to orange or red indicates the production of hydrogen cyanide by the desired isolate.

Biocontrol activity of endophyte symbiont bacteria against pathogen

Bacterial plant pathogens, *Pseudomonas* syringae PV. Syringe strain previously identified as a broad- and narrow-host range bacteria, respectively, were chosen as indicators. 300 µl of suspension (2 × 108 [9]CFU /ml) from bacterial pathogen *Pseudomonas* syringa was spread on a nutrient agar plate and kept at room temperature for 5 min. Then, sterile paper discs pre-immersed in the suspension of endophytic symbiont bacteria (concentration of about 108 CFU/ml) spotted on the pathogen-inoculated plates. Plates incubated at 28°C for 48–72 h and the width of the inhibition zones surveyed. Sterile water located in the plates with the pathogen was done as a control[26] The operation was duplicated twice with four replications.

Inoculation

Four groups of annual seedlings in pots containing soil of Kermanshah *oak* forests by selected bacterial samples from the initial screening of *Streptomyces* sp., *Pseudomonas* sp. *Bacillus* sp.) were inoculated. Seedlings inoculated with 15 ml of CFU/ ml (3×10^8) bacterial suspension (prepared using McFarland Standard No. 1). Also, Negative control samples inoculated with 0.1% saline[27]. Seedlings were

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0.4 g, ZnSO4 • 7H2O: 0.12 g, H3BO3: 1.4 g, Na2MoO4 • 2H2O: 1.0 g, MnSO4 • H2O: 1.5 g, Distilled water: 1.0 L, Vitamin solution: Biotin: 10 mg, Pyridoxol HCL: 20 mg, Distilled water: 0.1 L.

[8]28g Skim milk powder, 5g Casein enzymic, 2.5g hydrolysate Yeast extract, 1 g Dextrose, 15g Agar, Final pH (at 25°C)

[9]colony-forming unit

harvested three months after the bacteria were fixed and inoculated. Various vegetative parameters including fresh weight, leaf dry weight, stem, the root measured, and leaves measured after imaging with image j software. Seedling height, root length was measured. Finally, the ratio of fresh weight to shoot weight calculated. The current biomass weight (root, shoot, and leaf weight) was also calculated[28].

Analysis

Data analysis was performed using GraphPad Instate prism 8.1, and a comparison of means made with LSD test at the 1% level. By ANOVA-ONE Way test and Dunnett's test. Results were analyzed with 95% confidence level (P <0 · 05, P <0 · 001, P <0 · 0001).

Results:

Identification of isolates

Based on preliminary screening tests of 32 microbial strains (Table 1), soluble phosphate bacteria into three genera (*Streptomyces* sp., *Pseudomonas* sp. Bacillus Sp.) selected for subsequent experiments. Table 2 presents the results of survival variance, longitudinal, and diameter growth of *oak* seedlings. Results showed that two genera of *Streptomyces* sp., *Pseudomonas* sp. and the genus *Bacillus* sp., were identified. Among the 40 strains collected, only four could solubilize HAP. Of these four isolates, showing the largest halo zones and selected for further investigations[29].

Measuring of PGP[10]

As can be seen from the details in Table 2, the biosynthesis of indole acetic and gibberellic acid hormones was different among the isolates. Among the isolates were treated with *Streptomyces* sp.2Q and *Pseudomonas* sp. Qb accounted for the highest production of 84.11 and 61.14 µg /ml, respectively. *Streptomyces* sp.1Qb and *Bacillus* sp. Qb Strains subjected to the creation of these two hormones. *Streptomyces* sp.1Qb isolate treatment was associated with the highest production of gibberellic acid (76.34 µg /ml) (Table 2, fig1). All isolates were able to dissolve phosphate. Other indicators of growth promotion activity shown in Table 2.

biocontrol activity of the isolates

Among the tested strains of *Streptomyces* sp.1Qb with an average inhibitory zone diameter of 11.1 mm against *P*. syringae PV. The syringe has the highest inhibitory effect on this plant

[10] Plant growth-promoting

pathogen. *Bacillus* strain had no inhibitory effect on pathogen growth. Both *Streptomyces* sp.2Qb & *Pseudomonas* sp. Qb and *Streptomyces* sp.2Qb vessels were next in growth with 9 and 8 mm diameter inhibition zone, respectively (fig 2 a, b).

Biomass Treatment

Among the selected isolates, co-treatment of *the Bacillus* and *Streptomyces* group had significant effects on seedlings viability and strains of *Bacillus* sp. Qb, *Streptomyces* sp. Qb2, *Streptomyces* sp. Qb2 were ranked respectively. The results of *Pseudomonas* isolate on seedling viability were not significant (Table 4).

3.5 Effects on diameter and length of *oak* seedlings

According to the data, the effects of on seedling diameter by variance test and Dunnett's experiment showed that co-treatment with *Bacillus-Streptomyces* isolates had the highest mean diameter increase among other isolates (*Bacillus*, *Streptomyces* ($P < 0.001$), and *Pseudomonas* isolates had no significant effect on average diameter of seedling growth (fig3a, b; Table 3a, b; Table4, Table 5a, b).

seedling growth

Table 4 shows the concordance of the *Streptomyces* sp.2Qb & *Pseudomonas* sp. Qb had a significant effect on the root, stem, and leaf growth in comparison to the control (Table 4; fig 3a. b). Treatment with *Streptomyces* and *Pseudomonas* isolates and co-treatment also had a significant effect on root dry weight (fig3a; Table 5a, b). Treatment of *Bacillus*, *Pseudomonas,* and *Streptomyces* isolates alone did not have a substantial impact on leaf growth parameter, but also co-treatments of *Streptomyces* sp.2Q & *Pseudomonas* sp. Q on leaf dry weight of seedlings compared with control was significant (Table 5b).

Discussion

In this research, symbiotic bacteria of *oak* trees of Kermanshah have been studied to identify their diversity, dispersal, and ability to controlling pathogens and their role in promoting host growth. Dochhil et al. (2013) [30]described the plant growth enhancement and higher seed germination percentage by the employment of two *Streptomyces* sp. isolated from *Centella* Asiatica. Also, these strains estimated for the production of a plant growth promoter, indole acetic acid (IAA), which found in much higher concentration as 71 g/ml and 197 g/ml.

The isolates of the genus *Nocardiopsis* presented the highest IAA production ability among all

other *actinomycete* genera[31] . In the field trials conducted by[32], *Actinoplanes* campanulatus, *Micromonospora* chalcea, and *Streptomyces* spirals were applied individually and in aggregate to cucumber seedlings, which enhanced plant growth and yield.

Gangwar et al. (2014)[33] also found *actinobacteria*, mostly *Streptomyces* sp., capable of producing IAA. Plant growth promoters built within the range of 9.0–38.8 mg/ml.

El-Tarabily et al. (2010) applied endophytic symbiont *Actinoplanes* campanulatus, *Micromonospora* chance, and *Streptomyces* spiralis to cucumber seedlings. As it reduced seedling damping-off as well as root- and crown- rot of mature cucumber plants caused by *Pythium* aphanidermatum successfully, the authors suggested that these strains of endophytic symbiont *actinobacteria* employed as biological control agents.

The low content of free phosphorus in soil due to the formation of insoluble metallic complexes is a principal limiting factor in agriculture[34]. Microbes [35]in individual belonging to *actinobacteria* such as *Streptomyces*, *Micrococcus*, and *Micromonospora* [34, 36], *Kitasatospora* [37], and *Thermobifida* [38], are well characterized for their capability to solubilize phosphate. The strains of *Micromonospora* aurantiaca and *S.* Griseus are reported positive for overcoming damping-off caused by *Pythium* ultimum[34], and are also known for rock phosphate solubilization, indicating the importance in biological control under nutrient-deficient soils. The phosphate-solubilizing activity of *actinobacteria* is correlated to organic acid and siderophore production [34, 39].

The performance of *Streptomyces* strains in plant growth improvement has studied in various subjects [32, 36, 40, 41]. The inoculation of *actinomycetes* into the *Arabidopsis* rhizosphere is found to promote growth[42]. Increase raises by *actinomycetes* have been correlated with root elongation, whereas for endophytic symbiont strains such effects are generally attributed to plant growth regulators production, leading to better seedling growth and protection against tissue damage[32, 41, 43]. Additionally, growth promotion is also associated with siderophore production and phosphate solubilization (Hamdali et al., 2008b), and nitrogen fixation[41, 44]. Researchers isolated endophytic symbiont *actinomycetes* with antibacterial activity [45, 46], Recent studies show that the distribution of *actinomycetes* in different organs: leaves, roots, and stems of the same species[47]. The outcomes of this investigation reveal that the endophytes of Kermanshah *oak* trees are less diverse than similar studies,

and *Streptomyces*, *Pseudomonas*, and *Bacillus* are the dominant species (Table 1).

Separation of endophytic symbiont *actinobacteria* depends on various constituents, which comprise host plant species, age, and type of tissue, geographic and ecological distribution, sampling season, surface sterilant, particular media, and culture requirements [48, 49].

Dochhil et al. (2013), from the root, stem, and leaves of *Centella* Asiatica isolated *Streptomyces* sp. that produced Indole acetic acid (IAA) In a complex micro-ecosystem[50]. In this research, we studied the impact of growth-promoting bacteria (phosphate solubilizing ability) isolated from *oak* trees on the growth and stability of *oak* species and, consequently, increase the possibility of their establishment and survival[51]. It has been published that the amount of the bacterial growth-promoting effect on the bacterium and its population, plant-bacteria composition, plant genotype, type of parameters studied, and environmental conditions depend[52].

In most cases, treatment with growth-promoting bacteria increases viability, primarily if inoculation is carried out in seedlings, and then large-rooted offspring are transferred to the target area[28]. In another study, the effect of the type of growth-promoting bacteria on growth rate based on dry weight gain has emphasized[53].

This research is in agreement with the results of this study on the effect of selected isolates on dry weight growth (table5, fig.3). Effect of Inoculation on height and collar diameter increase by growth-promoting bacteria have reported in previous studies. The findings of this study regarding the effect of growth-promoting bacteria on height are consistent with the results of others regarding the direct relationship between growth with genus and species of bacteria[54]. Increased longitudinal and diameter growths of *Eucalyptus* species have been reported using *Pseudomonas* and *Bacillus* inoculation[55]. The effect of *Streptomyces*, *Pseudomonas*, and *Bacillus* isolates on different parameters of *oak* in the present study is significant and is consistent with other researchers. Based on the results of various studies showing the effect of inoculation on longitudinal and diameter extension, the outcomes of the instant research prove the role of bacterial endophyte growth (plant hormone production and root uptake) (Table 2 and Figure 2 a, b).

According to the events of the instant study, in comparison with other similar studies[56], the role of *Pseudomonas* and *Bacillus* in the growth effect of filamentous *actinobacteria* in growth promotion is

determined, especially in co-treatment conditions of *Streptomyces* and *Bacillus* isolates. The viability of *oak* seedlings was remarkable (Table 4 and Figure 3). Overall, it seems that the role of *Pseudomonas* and *Streptomyces* isolates in terms of growth parameters and production of different hormones on *oak* species of Dalahoo station. Selected isolates either in a single treatment or in co-treatment as biocontrol agents (*Streptomyces* sp. Qb1) and growth stimulator (*Streptomyces* sp. Qb2 & *Pseudomonas* sp. Qb) Kermanshah *oak* trees and control their deterioration in conservation programs and the restoration of such *oak* habitats are introduced (fig1, table 4& 5a, b).

Conclusions

Commented [PW1]: The part of *Conclusions* is optional.

The result of the present study help us to manage and control of plant pathogens and reforestation. Especially when different pathogens cause oak degradation, biological control is one of the most sustainable ways to combat the decline of Kermanshah *oak* trees. Description of selected *Streptomyces* isolates of *oak* trees of Zagros region and characterization of their metabolites recommended.

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Figure legends:

Figure 1

Production of Indole acetic acid and Gibberellic acid by endophytic symbiont strains. Every value signifies the mean of three replicates. Means with the same report are not significantly different at the 1% possibility level.

Figure 2

Fig. 2a: Width of the repression area produced by endophytic symbiont strains. (A) *P.* syringae 1: *Streptomyces* sp. Qb1, 2: *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb, 3: *Streptomyces* sp.2Qb, and 4: Bacillus sp. Qb strains.

Fig. 2b: Antagonistic activity of endophytic symbiont bacterial strains against bacterial plant pathogens, P. s. PV. Syringe under in vitro condition. 1: *Streptomyces* sp. Qb1, 2: *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb, 3: *Streptomyces* sp.2Qb, and 4: Bacillus sp. Qb strains. The data are averaged over three replications ± SE. Data analyzed by ANOVA and post hoc Duncan test < 0.05. Means by the parallel line are not significantly different.

Figure 3

Fig. 3a: Comparison of mean bacterial treatments on root dry matter percentage (P <0.05, 0.001, 0.0001, respectively) showed significant differences at 99% and 95% confidence level and ns, respectively.

Fig. 3b: Comparison of mean dry weight percentage of the stem in the presence of different isolates compared to the control group.

Table 1. Biochemical tests to identify isolated bacteria(Mac Faddin, 1976).

Test	***Streptomyces* sp.2Qb**	***Streptomyces* sp.1Qb**	***Bacillus* sp. Qb**	***Pseudomonas* sp.Qb**
Gram Test	Gram Positive	Gram Positive	Gram positive	**Gram negative**
Morphology	Filamentous	Filamentous	Bacillus	**Bacillus**
Oxidation	-	-	-	+
Catalase	+	+	+	+
Motility Test	Non-Motile	Non-Motile	Motile	**Motile**
Glucose	Variable	Fermentative	Variable	**Oxidative**
Methyl Red (MR) test	+	+	-	-
Voges–Proskauer test	+	+	-	-
Citrate	-	+	+	+
Nitrate	-	+	+	-
Indole	-	-	-	-

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Table 2. Plant growth potential properties of Endophytic symbiont strains oak trees and co-treatment.

Strain	P soluble (mg L^{-1})			N.fixation	HCN Pr.	Growth inhibition of *P.syringae*
Pseudomonas sp.Qb	82 ± 1.6[a]	56.22± 1.61[a]	32.11	+	-	-	+
Bacillus sp. Qb	72 ± 1.8[a]	52.21± 1.91[a]	54.22	-	-	-	_
Streptomyces sp.1Qb	93 ± 1.9[a]	58.12 ± 2.31[a]	76.34	+	+	+	+
Streptomyces sp.2Qb	95 ± 1.9[a]	44.34± 2.01[a]	58.12	+	+	+	+
Co-culture mixture of *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb	105 ± 1.1[a]	64.16± 1.72[a]	44.23	+	+	+	+

[a] Numbers given are the average of triplicate experiments ± SD. (P <0.05 *, ** P <0.001)

Table 3:

Tab. 3a: Comparison of mean diameter biomass of oak stems. Tab. 3b: Comparison of mean diameter biomass of oak seedlings.

3a:

Dunnett's test	**P-value**	**Conf.**
Control & *Pseudomonas* sp. Qb	0.9986	**Ns**
Control &. *Streptomyces* sp. Qb1	0.0025	**
Control & *Streptomyces* sp. Qb2	0.0018	**
Control &*Bacillus* sp. Qb	0.0007	***
Control & Co-treat *Bacillus* Sp.+ *Streptomyces* sp.	<0.0001	****

P <0.05, 0.001, 0.0001, respectively, were significant at 99% and 95% confidence level and ns respectively not significant.

3b:

	P-value	**Conf.**
Control vs. *Pseudomonas* sp. Qb	0.2038	**ns**
Control vs. *Streptomyces* sp. Qb1	0.0444	*
Control vs. *Streptomyces* sp. Qb2	0.0090	**
Control vs. *Bacillus* sp. Qb	0.0057	**

P <0.05, P <0.0001, respectively, were significant at 99% and 95% confidence level and ns respectively not significant.

Table 4. Results of species treatment based on root, stem, and leaf dry matter of oak.

Bacterial treatment	**Dry weight percent**		
	Root	Stem	**Leaf**
Pseudomonas sp.Qb	57±1/0	79.2±/3.1	**57.6±/1.5**
Bacillus sp. Qb	65.4± 47/ 0	70.6±/2.6	**43.1±/2.4**
Streptomyces sp.1Qb	80.6 ± 1/0	80.2±3.1	**59.4±/1.49**
Streptomyces sp.2Qb	86.13 ± 3/0	81.7 ±3.4	**60.2±/3.1**
Co-culture mixture of *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb	89.27 ± 2/0	84.2±3.2	**63.9±/4.5**
Control	60.6	70.9±۲،۳	**42.9±3.55**

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Table 5

Tab. 5a: Comparison of the mean dry weight of stem. Tab. 5b: Comparison of the mean dry weight of leaf seedlings between control and selected isolates.

5a:

Dunnett's test	**P-value**	**Conf.**
Control vs. *Pseudomonas* sp. Qb	0.9997	**No**
Control vs. *Streptomyces* sp. Qb1	0.3273	**No**
Control vs. *Streptomyces* sp. Qb2	0.9985	**No**
Control vs. *Bacillus* sp. Qb	0.3102	**No**
Control vs. Co-treat *Pseudomonas* sp.+ *Streptomyces*.sp. Qb2	0.0015	**Yes**

5b:

Dunnett's test	**P-value**	**Conf.**
Control vs. *Pseudomonas* sp. Qb	<0.0001	****
Control vs. *Streptomyces* sp. Qb1	<0.0001	****
Control vs. *Streptomyces* sp. Qb2	<0.0001	****
Control vs. *Bacillus* sp. Qb	0.9997	**ns**
Control vs. Co-treat *Pseudomonas* sp.+ *Streptomyces*.sp. Qb2	<0.0001	****

P <0.05, 0.001, 0.0001 Significant differences at 99% and 95% confidence level and ns respectively were not significant, respectively.

FIGURE 1: Production of Indole acetic acid and Gibberellic acid by endophytic symbiont strains. Every value signifies the mean of three replicates. Means with the same report are not significantly different at the 1% possibility level.

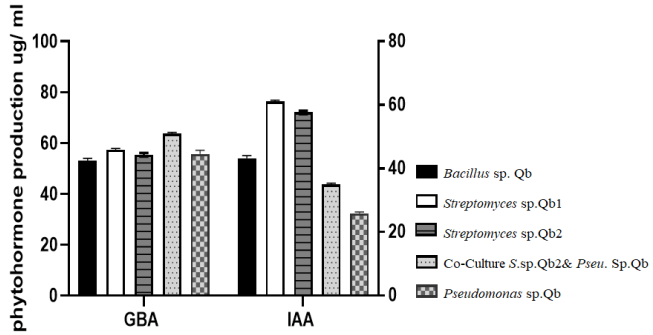

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FIGURE 2: **Figure 2**

Fig. 2a: Width of the repression area produced by endophytic symbiont strains. (A) *P*. syringae 1: *Streptomyces* sp. Qb1, 2: *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb, 3: *Streptomyces* sp.2Qb, and 4: Bacillus sp. Qb strains.

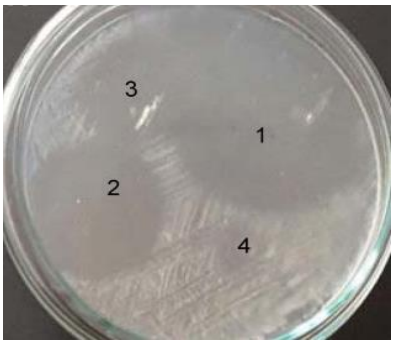


Fig. 2b: Antagonistic activity of endophytic symbiont bacterial strains against bacterial plant pathogens, P. s. PV. Syringe under in vitro condition. 1: *Streptomyces* sp. Qb1, 2: *Streptomyces* sp.2Qb & *Pseudomonas* sp.Qb, 3: *Streptomyces* sp.2Qb, and 4: Bacillus sp. Qb strains. The data are averaged over three replications ± SE. Data analyzed by ANOVA and post hoc Duncan test < 0.05. Means with the same report are not significantly different.

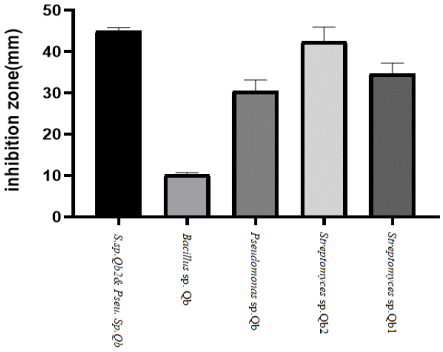

inhibition zone(mm)
50
40
30
20
10
0
Strp Qb2& Pseu. Sp. Qb
Bacillus sp. Qb
Pseudomonas sp. Qb
Streptomyces sp. Qb2
Streptomyces sp. Qb1

FIGURE 3: **Figure 3**

Fig. 3a: Comparison of mean bacterial treatments on root dry matter percentage (P <0.05, 0.001, 0.0001, respectively) showed significant differences at 99% and 95% confidence level and ns, respectively.

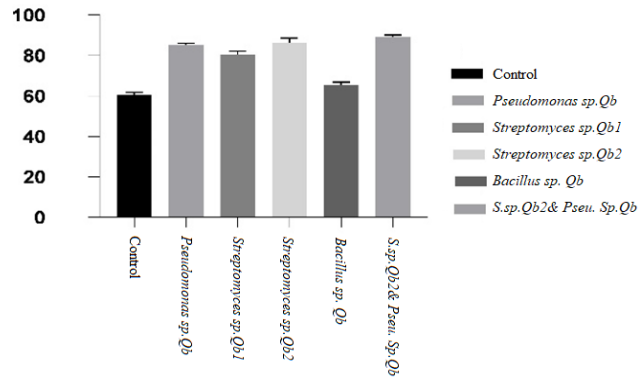
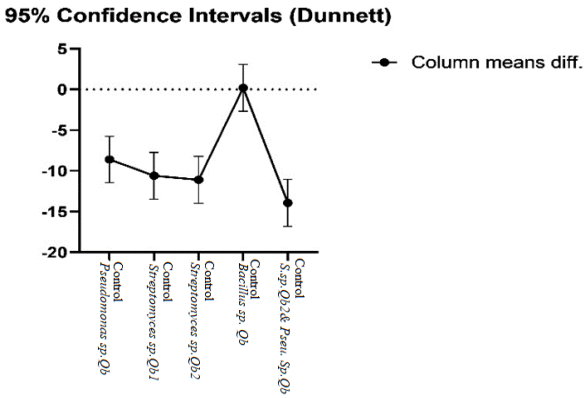


Fig. 3b: Comparison of mean dry weight percentage of the stem in the presence of different isolates compared to the control group.



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