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Molecular Phylogenetics of Microbial Endophytes Endowed with Plant Growth-promoting Traits from *Populus tomentosa*

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ENDOPHYTIC microorganisms are used as substitutes for chemical fertilizers and pesticides for increasing plant growth and productivity. In this study, we isolated 90 bacterial and 64 endophytic fungal isolates from the roots, stems, and leaves of different genotypes of Chinese white poplar (*Populus tomentosa* Carr.). The isolated endophytes were screened for the production of plant growth-promoting parameters, such as indole-3-acetic acid, siderophores, and inorganic phosphate solubilization. Furthermore, the endophytes were assessed for their activities of hydrolytic enzymes, such as amylase, cellulase, and pectinase. *In vitro* tests were conducted to visualize root colonization for the most potent isolates. Furthermore, 16S rRNA sequence analysis indicated that strains 24RB, 33RB, 59SB, 85LB, and 88LB displayed similarities to *Enterobacter tabaci, Bacillus velezensis, Bacillus megaterium, Pantoea eucrina,* and *Bacillus aryabhattai* ; these strains are deposited in GenBank under accession numbers MN540932.1, MN559965.1, MN540915.1, MN541091.1, and MN540958.1, respectively. Based on nucleotide sequencing from the internal transcribed spacer of rDNA, fungal isolate codes 17RF, 37RF, 46SF, 48SF, 52SF, and 63LF were identified as *Lasiosphaeriaceae* sp., *Chaetomium globosum, Aspergillus niger, Peyronellaea* sp., *Talaromyces amestolkiae, and Alternaria* sp. and deposited in GenBank with accession numbers MN541090.1, MN541117.1, MN540962.1, MN540968.1, MN540956.1, and MN541096.1, respectively. In conclusion, these strains have the potential to be commercialized for agricultural purposes.

Keywords: Bacterial endophyte, Fungal endophyte, Plant growth-promoting traits, Phylogenetic analysis, *Populus tomentosa*.

Introduction

Poplar, a member of the Salicaceae family, is a valuable source of wood, and bioenergy and an environmental resource (Richardson, 2001). The western balsam poplar (*Populus trichocarpa*) (Tuskan et al., 2006), the desert poplar (*Populus euphratica*) (Ma et al., 2013), and the Chinese white poplar (*Populus tomentosa*) are among the many notable woody species in the *Populus* genus (Du et al., 2014). In Central and Northern China, *P. tomentosa* is widely scattered and cultivated, and is economically and ecologically significant (An et al., 2020). Additionally, poplar can make various symbiotic relationships with a wide variety of microorganisms associated with soil including endophytes, ectomycorrhizal and endomycorrhizal fungi, and nitrogen-fixing bacteria (Bonito et al., 2014).

Endophytes are symbiotic microorganisms that infiltrate the intracellular and intercellular regions of plants without affecting plant morphology or causing infection (Fouda et al., 2019). Bacteria and fungi are the most common and most studied endophytes, which include bacteria, fungi,

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archaea, and protists (Kumar & Dara, 2021). Eid et al. (2019) reported that endophytes increase plant development by producing phytohormones and sustaining plant growth during biotic and abiotic stress. They also create secondary active metabolites that safeguard plants from pathogenic microbes, and exoenzymes, which may aid in microbial colonization.

The endophytic production of phytohormones, such as indole-acetic acid (IAA), gibberellins, and cytokinins (Wulandari & Suryantini, 2019), represents a well-studied plant growth-promoting (PGP) strategy, which results in architectural and morphological modifications in host plants (Khan et al., 2012). IAA promotes the formation and elongation of plant roots, root hairs, and lateral roots, which leads to an increase in nutrient absorption and plant development (Hagaggi & Mohamed, 2020; Hussain et al., 2013). Other important features of endophytes include their capability to solubilize insoluble phosphates, produce siderophores, and supply nitrogen to host plants (Matsuoka et al., 2013). Diazotrophic endophytes (nitrogen-fixing) may transform dinitrogen gas into nitrogen (N) compounds, such as ammonium and nitrate, which can then be used by the plant for nitrogen metabolism (Santi et al., 2013)**.**

The phosphorus-solubilizing activity of soil microorganisms is considered the most essential feature supporting plant growth and nutrient absorption (Rodríguez & Fraga, 1999). Many bacteria and fungi provide soil with organic acids, which assist in the solubilization of complex phosphates and convert them into soluble forms for plant absorption. Most bacterial genera, such as *Rhizobium*, *Bacillus*, and *Pseudomonas*, and fungal genera, such as *Penicillium* and *Aspergillus*, are phosphate-solubilizing microorganisms (PSMs) (Yadav et al., 2018).

Siderophores are low-molecular-weight molecules produced by endophytes for absorbing iron and making it available to plants while protecting against pathogens (Yadav et al., 2018). Some forms of siderophores, such as hydroxymate, phenolate, and catecholate, have biocontrol characteristics (Rajkumar et al., 2010). Different types of enzymes, such as cellulase, amylase, chitinase, and pectinase were reported to be produced by different genera of bacteria and fungi (Khan et al., 2017). These enzymes have an active role in initiating host symbiosis, establishing an association with the host, and initiating extracellular hydrolase activity to combat infection from plant pathogens (Leo et al., 2016).

Thus, considering these advantages of endophytic microbes, we aim to accomplish the following goals with our study: (1) Isolation of endophytic bacteria and fungi from different genotypes of *P. tomentosa* (2) Estimation of their potential for plant growth promotion, and (3) Molecular identification and selection of the most potent isolates.

Materials and Methods

Plant sampling and isolation of microbial endophytes

To isolate endophytic bacteria and fungi, we used three genotypes of 1-year-old *P. tomentosa* plants that were grown in a greenhouse at Beijing Forestry University, Beijing, China (40°0′N, 116° 20′ E). The samples were dissected into roots, stems, and leaves and washed well using tap water to get rid of sticky soil. After adequate washing, the plant parts were sliced into 0.5 to 1cm segments and superficially sterilized using 70% ethanol and 2% sodium hypochlorite for 30 s and 5min, respectively. Then the samples were washed two times with sterilized distilled water under a laminar flow hood (Elbeltagy et al., 2000). For bacterial isolation, the sterilized parts were ground with 0.8% saline solution and quartz sand and then diluted decimally in 0.8% saline solution. To ensure that the isolated strains were from inside the root (endophytes), sterility checks were performed. The last dilutions were spread on nutrient agar**,** tryptic soy, Luria–Bertani (LB) (Bric et al., 1991), and King agar media (King et al., 1954). For fungal isolation, surface-sterilized tissues were placed on potato dextrose agar (Manual, 1984), Czapek–Dox agar (Raper & Fennell, 1965), malt extract agar (Galloway & Burgess, 1950), and Melin–Norkrans agar (Marx, 1969) media, all of which were amended with 50mg/L of chloramphenicol to prevent bacterial growth and 35mg/L of Rose Bengal to inhibit the growth of fast-growing fungi (Numponsak et al., 2018). Bacterial and fungal plates were sealed with parafilm and incubated at 37°C for 48h and 28°C for 7d, respectively. After the incubation period, pure cultures were preserved on slants at 4°C for further study.

Assessment of PGP traits of endophytes

Indole-3-acetic acid production

For the determination and quantification of IAA production by endophytes, the bacterial isolates were inoculated into Luria broth (LB) media and fungal isolates were inoculated into Czapek-Dox liquid medium. Both media were supplemented with 1mg/mL L-tryptophan. Then, cultures were incubated at 28 ± 2°C, with continuous shaking at 125rpm for 2 and 5d for bacteria and fungi, respectively. Nearly 2mL of culture filtrate was centrifuged at 15000rpm for 1min, and IAA production was detected in the filtrate using Salkowski's reagent (7.5mL 0.5M $FeCl_3.6H_2O$, 150mL concentrated H_2SO_4, and 250mL distilled water). A 1mL aliquot of the supernatant was added to 2mL of Salkowski's reagent. The mixture was incubated in the dark for 20min at room temperature (Gordon & Weber, 1951). The appearance of a pinkish-red color confirmed the presence of IAA. Absorbance was measured using a spectrophotometer at 530nm. The concentration of the IAA produced by microbial endophytes was determined from the standard curve of a pure solution of IAA (Sarwar et al., 1992).

Phosphate solubilization

Qualitative estimation

The microbial isolates were tested for P-solubilization as follows: Pikovskaya's medium (glucose 10g/L; $Ca_3(PO_4)$ 2.5g/L; $(NH_4)_2SO_4$ 0.5g/L; NaCl 0.2g/L; $MgSO_4.7H_2O$ 0.1g/L; KCl 0.2g/L; $FeSO_4.7H_2O$ 0.002g/L; yeast extract 0.5g/L; $MnSO_4.2H_2O$; 0.002g/L; agar 15g/L; and 1L dis. H_2O) was prepared, and bromophenol blue was added as an indicator. Microbial isolates were inoculated into the medium and incubated for 5d. Uninoculated Pikovskaya's medium was used as a control. The ability of endophytes to solubilize phosphate was demonstrated by the creation of yellow zones around colonies because of the use of tricalcium phosphate (Jasim et al., 2013).

Quantitative assessment

For quantitative analysis of phosphate solubilization, positive phosphate solubilizer isolates were transferred to an NBRIP broth medium (Nautiyal, 1999) and then incubated for 7d at 28°C. The cultures were subjected to centrifugation at 5000rpm for 20min, and the phosphorous concentration in the culture supernatant was detected using para molybdenum blue (Dawwam et al., 2013). The color was assessed using a spectrophotometer at 600nm (Naik et al., 2009). A standard curve was established using potassium dihydrogen phosphate (KH_2PO_4) solution for quantitative determination.

Siderophore production

Siderophore production was checked using the Blue agar medium with chrome azurol S (CAS) and hexadecyltrimethylammonium bromide as indicators (Schwyn & Neilands, 1987). Bacterial and fungal isolates were inoculated onto CAS agar plates and incubated at 28°C in the dark for 2 weeks. Siderophore production was confirmed through the formation of yellow–orange halos around the colonies.

Nitrogen fixation

The nitrogen-fixing ability of bacterial isolates was assessed qualitatively by culturing bacteria in nitrogen-free (NF) medium, containing (g/L) 20g mannitol, 0.2g K_2HPO_4, 0.2g NaCl, 0.2g $MgSO_4.7H_2O$, 0.1g K_2SO_4, 5g $CaCO_3$, and 20g agar, using the spot inoculation method (Khoury et al., 2015). After a 48h incubation period at 28°C, the growth of the isolates was examined.

Screening for extracellular enzymes

Bacterial endophytes were inoculated into mineral salt media with the tested substrates to evaluate their production of extracellular enzymes. Furthermore, 5 mm endophytic fungal plugs were cultured on yeast-malt (YM) agar media (YM: 10g/L glucose, 5g/L peptone, 3g/L yeast extract, 3g/L malt extract, 1.5% agar [pH 6.7]) amended with dissolved and specific symptomatic substrates. Amylase, cellulase, and pectinase activities of endophytes were evaluated by growing the endophytic isolates on media supplemented with 1% of soluble starch, carboxy-methylcellulose, and pectin, respectively. The formation of a clear zone was detected after adding iodine, Congo red, and iodine–potassium iodide solution for amylolytic, cellulolytic, and pectinolytic activities, respectively (Fouda et al., 2015).

Root colonization assay

Shoot tips of *P. tomentosa* LM50 were sterilized, cut into 2cm long pieces, and placed in a magenta box containing 80mL of tissue media (Murashige & Skoog basal medium (Murashige & Skoog, 1962). After 1 week,

the bacterial (10^7 CFU/mL) and fungal (10^7 spore/mL) suspensions. were inoculated into *P. tomentosa* LM50 cultures. The attachment of endophytic microorganisms with the root was confirmed using 2,3,5-triphenyl-tetrazolium chloride (TTC) staining. Briefly, 0.15g TTC was mixed with sterile 50mM malate–potassium phosphate buffer (100mL of 50mM potassium phosphate buffer [pH 7] and 0.0625g malic acid) under aseptic conditions. Surface-sterilized plant segments were dipped in 20mL of sterile TTC solution and incubated overnight at 30°C (Jha et al., 2011). The sections were examined under the OLYMPUS DP72 microscope.

Molecular identification

Based on previous results, five bacterial (24RB, 33RB, 59SB, 85LB, and 88LB) and six fungal (17RF, 37RF, 46SF, 48SF, 52SF, and 63LF) isolates were chosen for molecular identification. Amplification and sequence analysis of the bacterial 16S rRNA gene and the fungal internal transcribed spacer (ITS) rDNA regions were used for molecular identification. Fungal DNA extraction was performed using the EZgene ™ Fungal DNA Miniprep kit, according to the manufacturer's instructions. Direct colony PCR was conducted on bacterial isolates. The 16S rRNA of the strain was amplified using forward 16S-F: 5′-AGAGTTTGATCCTGGCTCAGAACGAACGCT-3′ and reverse 16S-R: 5′-TACGGCTACCTTGTTACGACTTCACCCC-3′ primers. The amplification of the fungal ITS region was performed using forward ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and reverse ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (White et al., 1990). The PCR product was detected by preheating at 94°C for 5min; then, 35 cycles of 1min at 94°C, 55°C for 40s, and 72°C for 1min; and final extension of 72°C for 10min. The PCR products were checked for expected size on 1% agarose gel and sequenced using the services of the service Beijing Ruibio Biotech, Co., Ltd., on the ABI3730XL DNA Analyzer.

Phylogenetic analysis of endophytic microbes

To identify the closest known taxa, nucleotide sequences were compared with nucleotide databases using the NCBI BLAST search program for the final identification of the microbial endophytes program. A phylogenetic tree was constructed by the neighbor-joining method using the MEGA7 software (http://www.megasoftware.net/), after pairwise alignments using ClustalW (Kumar et al., 2016). Internal branch stability was evaluated using 1,000 bootstrap replications.

Results

Distribution of endophytic bacteria and fungi within P. tomentosa tissues

To investigate the involvement of endophytic bacteria and fungi in promoting plant growth, we isolated up to 90 isolates of bacteria and 64 isolates of fungi from surface-sterilized tissues of the three genotypes of *P. tomentosa*. The distribution of bacterial and fungal endophytes varied among tissues. We obtained 41, 28, and 21 bacterial and 40, 15, and 9 fungal isolates from roots, stems, and leaves, respectively (Table 1). The results revealed that endophytic bacteria and fungi were found in greater numbers in the roots than the stems and were least dominant in the leaves. Moreover, bacterial richness in different plant parts was higher than fungal diversity.

Direct PGP activities for microbial endophytes

Indole-3-acetic acid production

When testing the potential of isolates for IAA production, we observed that the supernatant of most turned red when incubated with Salkowski's reagent to form a positive-colored product, which was more intense in bacteria than fungi (Supplementary Fig. 1A and B). Our results revealed that among various bacterial isolates, IAA production ranged from 0.42±0.06 to 150.84±1.15µg/mL. Moreover, fungal isolates produced IAA in varying degrees, ranging from 6.16±0.98 to 81.4±1.2µg/mL (Supplementary Tables 1 and 2).

Qualitative solubilization of inorganic phosphate by endophytic bacteria and fungi

To assess the ability of endophytes to convert phosphorus from an insoluble to a soluble form, we tested them for phosphate solubilization. The formation of a yellowish halo because of phosphate utilization on solid media was considered a positive result. Our data revealed that 25.5% of bacterial isolates exhibited high phosphate solubilization, whereas 74.5% did not show any solubilization on solid media. Moreover, approximately 24% of the tested fungal isolates could solubilize phosphate. The other fungal isolates were unable to utilize phosphate (Supplementary Fig. 1B and E).

TABLE 1. Microbial distribution in different parts (roots, stems, and leaves) of three genotypes of *P. tomentosa.*

Genotypes	Bacterial endophyte distribution			Fungal endophyte distribution		
	Roots	Stems	Leaves	Roots	Stems	Leaves
Genotype1	15	14	11	16	10	6
Genotype2	12	8	7	11	3	3
Genotype3	14	6	3	13	2	0
Total	41	28	21	40	15	9

Quantitative phosphate solubilization by bacterial and fungal endophytes

To further test the isolates for phosphate solubilization quantitatively, we inoculated them into NBRIP liquid media. Bacteria gave darker blue color than fungi (Supplementary Fig. 1C and F). Results demonstrated that bacterial isolates solubilize phosphate in the range of 0.13–51.4µg/mL. For fungal isolates, the amount of soluble phosphate varied between 2.5±0.11 and 40.7±0.69µg/mL (Supplementary Tables 1 and 2).

Siderophore production

Siderophore production is a prominent feature of endophytes. Our data showed that in the 90 bacterial endophytes, only isolates 24RB, 33RB, 59SB, 85LB, and 88LB were strongly positive for siderophore production. By contrast, in the 64 fungal endophytes, only two isolates (46SF and 52SF) were strongly positive for siderophore production, which was indicated by a purplish-red formation. The remaining isolates did not show any siderophore production (Supplementary Tables 1 and 2).

Nitrogen fixation

Our findings revealed that only six bacterial isolates (18 RB, 21RB, 24RB, 35RB, 84LB, and 85LB) could survive in an NF medium, showing diazotrophic capability (Supplementary Table 1).

Indirect PGP features of microbial endophytes

Extracellular enzyme production

To investigate the indirect mechanism by which endophytes promote plant growth, we evaluated enzyme production by endophytes. Approximately 50% of bacterial isolates exhibited maximum production of amylase, 17% produced moderate amounts, and the others were unable to produce. For cellulase production, 58% of isolates showed the highest production, whereas 29% did not produce cellulase. Moreover, 30% of isolates produced the highest amount of pectinase, whereas 50% of the tested isolates did not show pectinase activity on solid media (Fig. 1A).

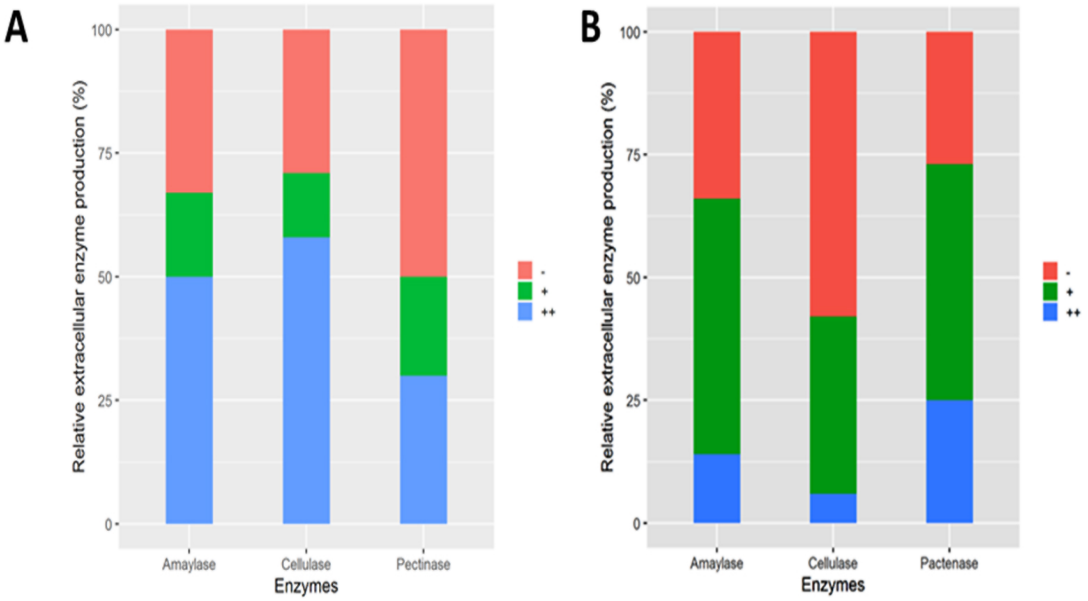

Fig. 1. Indirect plant growth-promoting activity of endophytes; (A) Relative extracellular enzyme production (amylase, cellulase, and pectinase) by endophytic bacterial isolates, (B) Relative extracellular enzyme production (amylase, cellulase, and pectinase) by endophytic fungal isolates

Regarding fungal endophytes, we found that approximately 14% of isolates exhibited the maximum production of amylase, whereas 52% showed moderate production. The other fungal isolates were unable to hydrolysis starch, giving negative results for amylase production. Regarding cellulolytic activity, 6% of the tested isolates were the highest producers of cellulase, although 36% of isolates showed low cellulase activity and the remaining isolates were unable to produce it. Approximately 25% of fungal isolates displayed the highest pectinase activity, whereas 48% produced average pectinase activity. The other isolates were unable to produce pectinase (Fig. 1B). Different bacterial and fungal isolates could degrade different substrates by producing specific enzymes on different media (Supplementary Table 3). These results were markedly obvious with the addition of different indicators (Fig. 2).

Selection of the most potent isolates

Based on our results, five bacterial isolates (24RB, 33RB, 59SB, 85LB, and 88LB) and six fungal isolates (17RF, 37RF, 46SF, 48SF, 52SF, and 63LF) have been chosen for their PGP activities (Table 2). Table 3 shows the most active, enzyme-producing bacterial and fungal isolates.

Endophytic colonization

The potential of some bacterial and fungal cells to proliferate into a large population in association with the roots is referred to as colonization (Fig. 3). The roots of inoculated plants appeared red because bacterial and fungal association reduces root TCC. By contrast, the roots of noninoculated plants (control) were colorless. Microscopically, the different microbes appeared as red cells after TTC staining. Bacterial and fungal colonies associated with the roots were seen as red spots because TTC only stains living cells (respiring); dead root cells remained colorless.

Molecular identification

Based on the results of the initial screening, we selected and identified the most active PGRB (24RB, 33RB, 59SB, 85LB, and 88LB) and the most active PGRF (17RF, 37RF, 46SF, 48SF, 52SF, and 63LF). Furthermore, 16S rRNA sequence analysis indicated that strains 24RB, 33RB, 59SB, 85LB, and 88LB displayed similarities to *Enterobacter tabaci*, *Bacillus velezensis*, *Bacillus megaterium*, *Pantoea eucrina*, and *Bacillus aryabhattai*. We have submitted the sequences for these strains in GenBank under accession numbers MN540932.1, MN559965.1, MN540915.1, MN541091.1, and MN540958.1, respectively. By contrast, ITS-rDNA sequences were PCR amplified and sequenced. The newly identified sequences were submitted in the GenBank database under the following accession numbers: MN541090.1, MN541117.1, MN540962.1, MN540968.1, MN540956.1, and MN541096.1, respectively. A BLAST search against the GenBank or proprietary fungal DNA databases showed that the rDNA sequence of fungal isolate codes 17RF, 37RF, 46SF, 48SF, 52SF, and 63LF displayed 100% similarity with *Lasiosphaeriaceae* sp., *Chaetomium globosum*, *Aspergillus niger*, *Peyronellaea* sp., *Talaromyces amestolkiae*, and *Alternaria* sp., respectively (Table 4). Figure 4 shows a phylogenetic tree clarifying the relationship between the selected isolates and other strains.

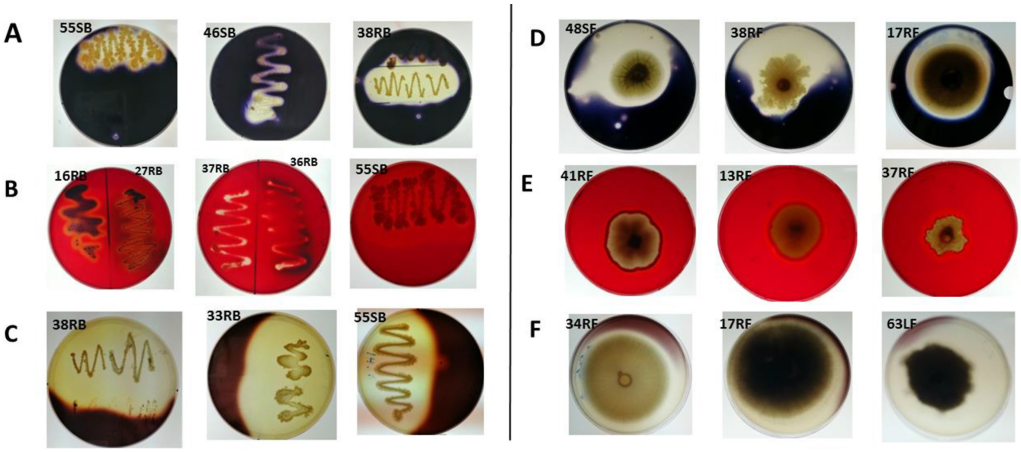

Fig. 2. Extracellular enzyme production by the most active bacterial endophytic producers on mineral salt and fungal endophytic producers on yeast-malt (YM) media supplemented with the indicated substrates; (A, B, and C) Amylase, cellulase, and pectinase production by endophytic bacterial isolates, respectively, (D, E, and F) Amylase, cellulase, and pectinase production by endophytic fungal isolates, respectively

TABLE 2. Different plant growth-promoting activities produced by the most potent isolates

Isolates code	IAA (µg/mL)	Phosphate solubilization		Nitrogen fixation	Siderophore production
		Quantitatively (µg/mL)	Qualitatively		
24RB	88.7±1.41	51.63±0.83	++*	+	++
33RB	45.4±0.96	19.41±1.24	+	ND	++
59SB	42.16±0.92	49.44±0.82	++	ND	++
85LB	53.1±0.79	46.18±0.85	++	+	++
88LB	44.12±0.57	34.08±1.23	++	ND	++
17RF	47.91±0.04	10.66±0.5	+	ND	-
37RF	81.4±1.2	8.7±1.3	+	ND	-
46SF	43.24±0.84	17. 58±0.84	+	ND	++
48SF	38.95±0.5	6.84±0.79	+	ND	-
52SF	33.66±0.05	40.7±0.69	++	ND	++
63LF	6.16±0.98	7.6±1.4	+	ND	-

*ND, +, and ++ indicate no, low, and high production, respectively. Values are means ± SE (n =3).

TABLE 3. Extracellular enzymes produced by the most potent isolates

Isolates code	Extracellular enzymatic activities		
	Cellulase	Amylase	Pectinase
24RB	+*	ND*	ND
33RB	++	++	++
59SB	++	++	+
85LB	++	ND	ND
88LB	++	++	++
17RF	+	++	+
37RF	+	+	+
46SF	++	+	++
48SF	+	+	+
52SF	+	+	+
63LF	+	+	++

*ND, +, and ++ denote no, low, and high production, respectively. Values are means ± SE (n= 3).

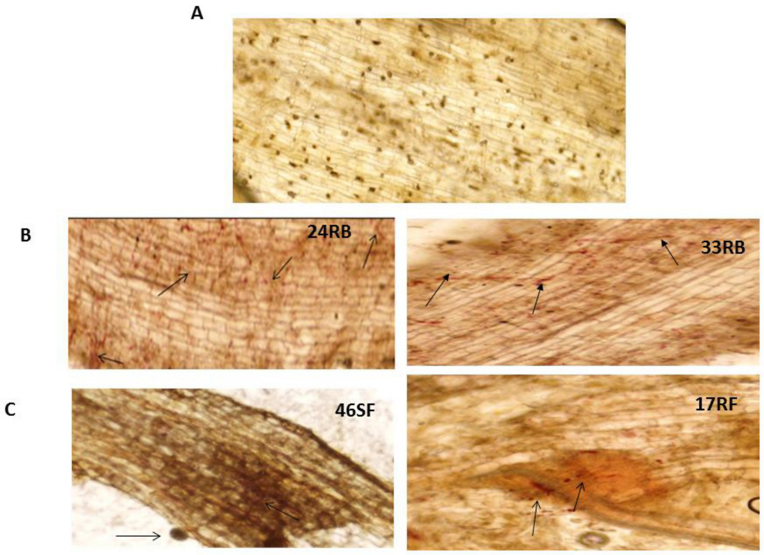

Fig. 3. Root colonization of *Populus tomentosa;* (A) Uninoculated root (control), (B) root colonized by bacteria, and (C) root colonized by fungi

TABLE 4. Molecular identification of the most potent endophytic microbial isolates

Isolates code	Fragment length (bp)	Most closely related taxa	Accession numbers
24RB	900	*Enterobacter tabaci*	MN540932.1
33RB	880	*Bacillus velezensis*	MN559965.1
59SB	880	*Bacillus megaterium*	MN540915.1
85LB	870	*Pantoea eucrina*	MN541091.1
88LB	894	*Bacillus aryabhattai*	MN540958.1
17 RF	504	*lasiosphaeriaceae* sp	MN541090**.1**
37 RF	543	*Chaetomium globosum*	MN541117.1
46 SF	570	*Aspergillus niger*	MN540962**.1**
48 SF	509	*peyronellaea sp.*	MN540968**.1**
52 SF	553	*Talaromyces amestolkiae*	MN540956.1
63LF	540	*Alternaria* SP.	MN541096**.1**

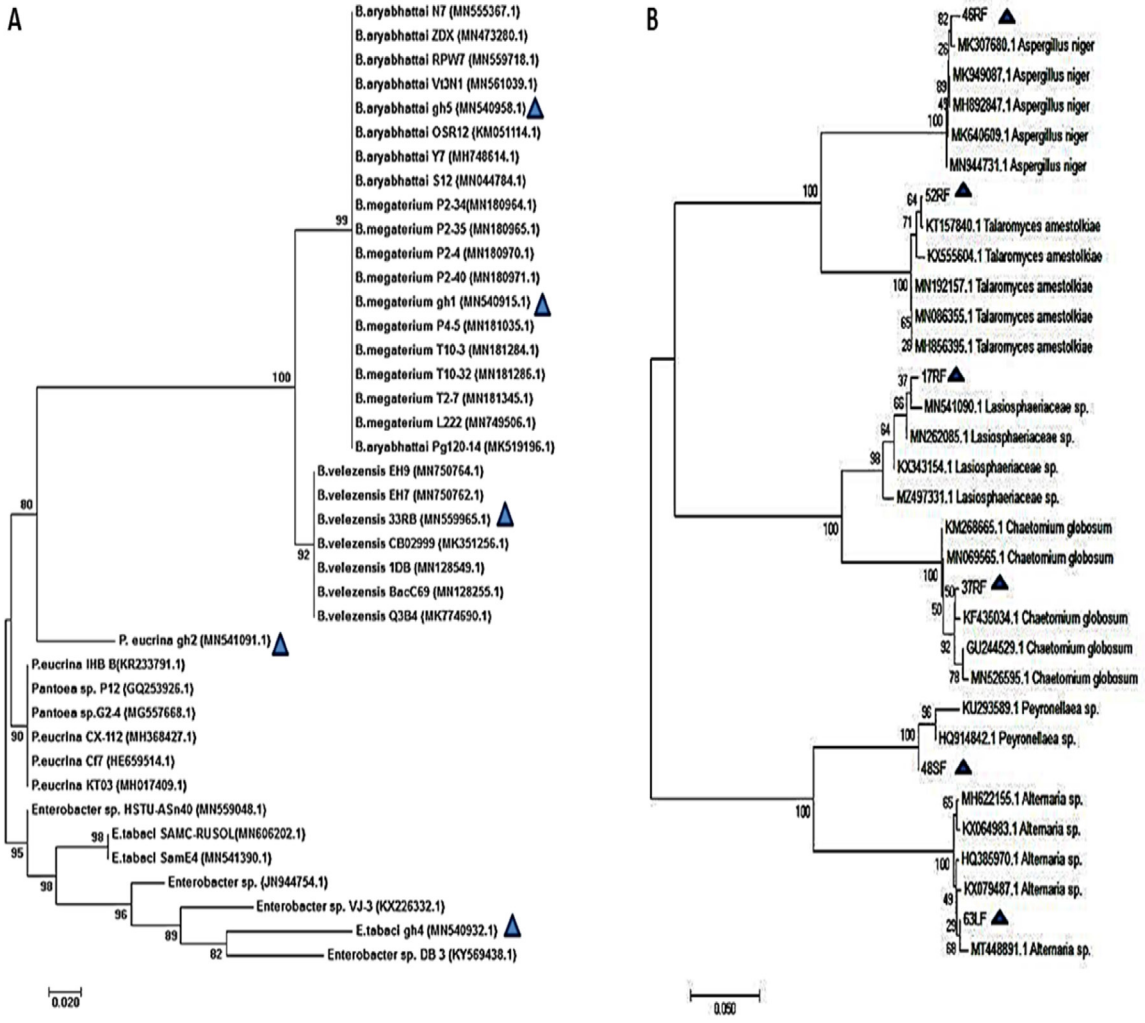

Fig. 4. Phylogenetic tree constructed using 16S rDNA sequences of (A) The most potent bacterial isolates and (B) ITS-rDNA sequences of the most potent fungal isolates using the neighbor-joining method [Evolutionary analyses were conducted using MEGA7]

Discussion

Endophytic microbes inhabit the internal parts of plants and promote their growth. Our study aimed to isolate endophytic bacteria and fungi from the different organs of *P. tomentosa* plants in relation to different PGP activities. Our data revealed that endophytic microbes are more predominant in the roots than in the stem, with the least amount present in the leaves. Similar results were reported by Zheng et al. (2017). The difference in endophyte assemblage in various tissues indicated that some microbial endophytes have an affinity for different tissue types. This attraction could be due to their ability to use or survive on a particular substrate (different tissue texture and chemistry) (Huang et al., 2008). Several additional investigations have found differences in colonization and isolation ratios, which accord with our findings (Naik et al., 2009; Lv et al., 2010).

Some endophytic microbes can synthesize IAA to increase plant growth upon colonization (Shi et al., 2009). The production of IAA by endophytic microbes may increase the growth of the host plant and increase the degree of protection against external adverse conditions by promoting cellular defense systems (Bianco et al., 2006). Thus, IAA production-based screening can be a useful technique for discovering beneficial microorganisms that influence plant growth (Govindarajan et al., 2007).

The capability of microorganisms for phosphate solubilization and mineralization in various culture media and with different phosphate sources has been reported in several studies (Pikovskaya, 1948; Nautiyal, 1999). Consistently, we found variations in the size of the solubilization zone in this study. This zone results from the secretion of substances, such as organic acids, by microorganisms that solubilize the P in the medium. The release of these substances leads to the generation of a translucent zone around the colonies, indicating their solubilizing capacity (Souchie et al., 2005).

PSMs have been studied extensively to determine their ability to solubilize low-solubility phosphorus in a liquid medium (Whitelaw, 1999). The secretion of organic acids, such as citric, oxalic, and lactic acids, is the primary cause of solubilization in a liquid medium (Khan et al., 2014). A reduction in hydrogen potential and cation chelation is linked to the production of organic acids by PSM during phosphorus solubilization (Nahas, 1996).

Siderophore production is another important feature of endophytes. The presence of an orange halo or purplish-red color surrounding colonies indicates iron chelation. This occurs because of siderophore production, which removes iron from the dye complex and changes the color of the medium from blue to orange (Schwyn & Neilands, 1987). Siderophore-producing microorganisms can chelate a limited amount of iron, thereby decreasing its availability for the growth of phytopathogens. Thus, they promote plant growth indirectly (Alexander & Zuberer, 1991). Isolates of gram-negative (*Pseudomonas* sp. and *Enterobacter* sp.) and gram-positive (*Bacillus* and *Rhodococcus*) bacteria are capable of producing siderophores (Tian et al., 2009). Moreover, fungal species, such as *Trichoderma harzianum*, *Penicillium citritum*, and *A. niger*, can produce siderophores and increase root and shoot length in chickpea (Yadav et al., 2011).

Our findings revealed that a few tested bacterial endophytes fixed nitrogen on NF media. Nitrogen fixation in rhizobial bacteria that are symbiotic with plant hosts has been studied. Some nitrogen-fixing endophytic bacteria have been isolated from trees such as poplar and willow (Doty et al., 2009). Bacterial endophytes, such as *Pantoea*, *Pseudomonas*, and *Serratia*, were reported as having nitrogen-fixing potential for other plants (Loiret et al., 2004).

Enzymatic activities are indirect mechanisms by which endophytes promote plant growth. Data revealed that most isolated endophytes produced amylase, cellulase, and pectinase. These enzymes perform hydrolytic functions that allow endophytes to penetrate plant tissue and establish a symbiotic relationship with the host plant. The enzymatic activities of endophytes provide their host plants with protection against pathogenic microorganisms through cell wall hydrolysis of pathogens (Glick, 2012). Cellulase was reported to trigger an abnormal hyphal morphology of pathogens, leading to the hyphal deformation and growth repression of pathogens (Shrestha et al., 2015). Although endophytes receive their nutrients from plants through the release of enzymes, their extracellular enzymatic

activities promote plant nutrition, indicating plant senescence through the degradation of proteins and polysaccharides (Choi et al., 2005).

The inoculating microbes are first attracted to the roots by chemotaxis and aerotaxis. Then, they colonized the roots, as evidenced by the appearance of red color. Pectinase and exopolysaccharide may play a vital role in the association between the host plant and microorganisms. In a study, bacteria reduced 2,3,5 triphenyl tetrazolium dichloride to red formazan in 3–4h, thereby helping detect *Azospirillum lipoferum* in surface-sterilized corn roots, sorghum, and wheat (Patriquin & Döbereiner, 1978). Similar results were observed by another study (Jha et al., 2011), which confirmed the adhesion and invasion of the isolated strains with paddy roots using 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining.

Conclusions

Both endophytic bacteria and fungi isolated from different tissues of *P. tomentosa* plants exhibited effective PGP activities, such as IAA production, inorganic phosphate solubilization, and siderophore production. Moreover, the endophytes exhibited indirect PGP traits, such as extracellular hydrolytic enzymes production. Thus, these endophytes are promising candidates for PGP, and their use as biofertilizers in agriculture is recommended for long-term sustainability.

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التقارب الجزيئي للكائنات النامية داخليا المتميزة بالصفات المعززة لنمو النبات ومعزولة من نباتات حور تومينتوسا

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يفضل استخدام الكائنات الدقيقة كمصدر بديل للأسمدة الكيماوية ومبيدات الآفات لتعزيز نمو النبات وإنتاجيته. في هذه الدراسة قمنا بعزل تسعين عزلة بكتيرية وأربعة وستين عزلة فطرية من جذور وسيقان وأوراق انماط وراثية مختلفة من الحور الأبيض الصيني *Populus tomentosa*. تم فحص النباتات الداخلية المعزولة بحثًا عن الصفات المعززة لنمو النبات مثل انتاج هرمون الإندول والسيدروفورز والقدرة علي اذابة الفوسفات غير العضوي. علاوة على ذلك، تم تقييم هذه العزلات لإنتاج الانزيمات المختلفة مثل السليوليزوالبكتينيز والأميليز. استخدمت الاختبارات في المعمل لتصوير استعمار الجذر للعزلات الأكثر فاعلية.

أشار تحليل تسلسل 16S إلى أن السلالات 24RB، 33RB ، 59SB، 85LB و 88LB أظهرت أوجه تشابه مع *Enterobacter tabaci* ، *Bacillus velezensis* ، *Bacillus megaterium* ، *Pantoea eucrina* و *Bacillus aryabhattai* وتم إيداع هذه السلالات في بنك الجينات تحت أرقام الانضمام MN540932.1, MN559965.1, MN540915.1, MN541091.1, MN540958.1 على التوالي. في حين أنه ، بناءً على تسلسل النوكليوتيدات (ITS) لـ rDNA ، تم تحديد رمز العزلات الفطرية 17RF و 37RF و 46SF و 48SF و 52SF و63LF على أنها Lasiosphaeriaceae sp, *Chaetomium globosum, Aspergillus niger, Peyronellaea sp., Talaromyces amestolkiae and Alternaria* sp. والمودعة في بنك الجينات تحت رقم الانضمام MN541090.1 و MN541117.1 و MN540962.1 و MN540968.1 و MN540956.1 و MN541096.1 على التوالي. في الختام، تؤكد هذه السلالات على فرصة تسويقها من أجل التنمية الزراعية المستدامة.