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Isolation and characterization of culturable endophytic plant growth-promoting *Bacillus* species from *Mentha longifolia* L.

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Abstract: Plant growth-promoting bacteria is one of the most popular promising alternative fertilizers for sustainable agriculture. Hence, it is preferred by agriculturalists today due to its nontoxic, eco-friendly, and affordable costs as well as its benefits on agricultural products. Among these microorganisms, *Bacillus* is a widely known and investigated genus in rhizosphere of plenty of agricultural products. In the current study, endophytic *Bacillus* strains were isolated from the root, stem, and leaves of healthy *Mentha longifolia* L. in Palandöken Mountain, Erzurum-Turkey and evaluated their plant growth promoting parameters such as nitrogen fixation, phosphate solubilization, IAA, siderophore, ACC deaminase, HCN production, and ammonia potential. According to the results, 12 *Bacillus* strains showed multiple plant growth promoting properties (PGPs). Moreover, molecular identification of the potential PGP *Bacillus* strains was carried out by using PCR with the universal primers for 16S rRNA gene region. These strains were containing 3 *Bacillus* sp. (ML7, ML17 and ML46), 3 *Bacillus simplex* (ML12, ML25 and ML43), 2 *Bacillus megaterium* (ML36, ML61), 1 *Bacillus muralis* (ML49), 1 *Bacillus aryabhattai* (ML55), 1 *Bacillus pumilus* (ML59), and 1 *Bacillus toyonensis* (ML63). In conclusion, these results demonstrated that endophytic *Bacillus* species from native medicinal plants have huge potential for being utilized as promising natural plant growth-promoter as biofertilizers, biostimulants, and bioprotectants in sustainable crop production due to their beneficial PGPs.

Key words: *M. longifolia* L., endopyhytic bacteria, *Bacillus*, 16S rRNA, plant growth promoting properties

1. Introduction

From ancient times until today, plants have been utilized as a pivotal resource for humans to meet their food and health needs. Especially, plants were thought of as a key solution to numerous diseases. Plants have been known since the first appearance of mankind on the earth that they played an important role in overcoming many diseases, and they are contributed to folk and modern medicine with their beneficial precursors for drugs since the past (Sahin et al., 2002; Zia-Ul-Haq et al., 2013; Anwar et al., 2019; Kesdek et al., 2020; Mollova et al., 2020; Subasi 2020). In this regard, numerous medicinal and aromatic plant families are revealed with commercial and medicinal targets. In these families, Lamiaceae (Labiatae) is known to contain plenty of medicinal and aromatic plants. The family consists of approximately more than 230 genera and around 7200 species (Singh et al., 2018). Among these genera, *Mentha* is one of the most favorable and popular medicinal and aromatic plants, which has an economically crucial role in food and health sectors because of its beneficial chemical components (Farzaei et al., 2017; Singh et al., 2018; Anwar et al., 2019).

The genus *Mentha* is commonly found in tropical and temperate regions of the world such as America Australia, Europe, Brazil, China, India, and South Africa, and it consists of more than 25 species with 30-60 cm long (Hajlaoui et al., 2008; Snoussi et al., 2015). *Mentha arvensis* L. (corn mint), *Mentha citrate* Ehrh. (bergamot mint), *M*entha x *piperita* L. (peppermint), *Mentha spicata* L. (spearmint) and *Mentha longifolia* L. (wild mint) are well known species for their positive properties with culinary, medicinal, aroma, therapeutic gastrointestinal disorders, respiratory disorders, infectious, and inflammatory diseases (Santana et al., 2016; Farzaei et al., 2017). Furthermore, the members of the genus contain plenty of components, which are utilized in food, cosmetic, and pharmaceutical products. Carvone, isomenthone, linalool, limonene, menthol, menthone, menthofuran, piperitenone, piperitenone oxide, and pulegone are well recognized chemical components of the genus (Santana et al., 2016; Hanafy et al., 2020).

Among the well-known economically important species of the genus, *M. longifolia* L. was reported as a herb with some various pharmacological properties such

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as antimicrobial, anticancer, antioxidant, gastrointestinal, and nervous system effects (Mikaili et al., 2013; Abbas and Nisar, 2020; Patti et al., 2020). Besides, *M. longifolia* L. is cultivated not only for the pharmaceutical industry but also for the food, cosmetic, and perfumery industries in many countries of the world because of its high economic value (Karla et al., 2005).

However, several pathogenic fungi and bacteria are restricted to the commercial production of medicinal and aromatic plants. For *Mentha* species, *Alternaria alternate* (leaf spot), *Erysiphe cichoracearum* (powdery mildew), *Nigrospora oryzae* (brown leaf spot), *Puccinia menthae* (rust), *Verticillium dahliae* (wilt), *Phoma stasserti* (stem rot), and *Rhizoctonia solani*, *Rhizoctonia bataticola* (root and stolon rot) have been reported as one of the most economically critical diseases occurred by fungal pathogens according to the literature (Karla et al., 2005; Vining et al., 2005; Dung et al., 2010; Singh et al., 2016; Farid et al., 2020). For example, the *Verticillium* wilt disease caused by *Verticillium dahliae* has hazardous effects on wild mint plants such as anthocyanescence, bronzing or curling of the apical leaves, chlorosis, necrosis, wilt, premature senescence, and stunting (Dung et al., 2010). Some fungicides have been utilized to control fungal diseases on *Mentha* pathogens (Karla et al., 2005). Also, chemical fertilizers containing nitrogen, phosphorus, and potassium have been commonly applied in previous studies to increase the production rate of the essential oil content of *M. longifolia* L. (Alsafar and Al-Hassan, 2009; Salehi et al., 2018; Al-Fredan and Al-Fadal 2019). However, the overutilization and unconscious usage of chemical-based fertilizers cause dangerous effects on the environmental problems because of their decreasing of soil fertility and microbial diversity, creates resistance between phytopathogens and insects, degradation of soil, reduction of crop productivity, and environmental pollution (Egamberdieva et al., 2015; Alaylar et al., 2018; Alaylar et al., 2019; Aeron et al., 2020; Alaylar et al., 2020a; Alaylar et al., 2020b). As an alternative approach, plant growth-promoting rhizobacteria (PGPR) have been evaluated as new, eco-friendly, efficient biotechnological tools that are required to enhance growth rate and productivity of crops including medicinal and aromatic plants (Rezaei-Chiyaneh et al., 2020).

Among these bacteria, endophytic microorganisms are recognized to be situated asymptomatically inside of the host plants at various stages of the life cycle and perform vital roles in the growth, development, and diversifications of the host (Golinska et al., 2015; Khan et al., 2017; Singh et al., 2017; Harrison and Griffin, 2020). It has been reported in previous studies that bacterial genera such as *Azotobacter*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas*, *Serratia,* and *Bacillus* etc. have demonstrated plant growth promoting properties (PGPs) (Kaki et al., 2013; Panchami et al., 2020). Also, these microorganisms have a beneficial function in the growth promotion of their host plants by fixing atmospheric nitrogen, potassium, zinc and phosphate solubilization, siderophore, ammonia, hydrogen cyanide, 1-aminocyclopropane-1-carboxylate (ACC), phytohormones (auxins, cytokinin, gibberellic acid) production, depletion of ethylene biosynthesis, increase in the nutrient uptake, supply tolerance to biotic and abiotic stresses and possess as biocontrol agents against to plant pathogens. Inside of these genera, *Bacillus* has critical roles compared with the other genera because of their endurance to harsh environmental conditions by spore formation. Therefore, plenty of *Bacillus* strains has been commercially revealed as biocontrol, biofertilizer, and biostimulant in agricultural practices nowadays (Lolloo et al., 2010; Kaki et al., 2013; Panchami et al., 2020).

Basically, endophytic microorganisms are located in internal tissues, roots, petiole, leaves, fruits, ovules, nodules, stem or seeds of plants and make useful effects on plants by producing substances such as hydrolytic enzymes (cellulase, pectinase, proteinase, xylanase), antimicrobials, and plant growth-promoting compounds (Dubey et al., 2020; Rana et al., 2020). According to the previous studies in the literature, numerous aromatic and medicinal plants have been investigated, and plenty of novel endophytic microorganisms were isolated from *Aloe vera*, *Armoracia rusticana*, *Dracaena cochinchinensis*, *Fagonia mollis,* and *Achillea fragrantissima* etc. (Salam et al., 2017; Vyawahare et al., 2019; Alkahtani et al., 2020; Egamberdieava et al., 2020).

Therefore, this study was performed to investigate the endophytic *Bacillus* strains medicinally and economically important plant wild mint (*M. longifolia* L.). Cultivable endophytic bacterial strains were isolated from wild mint plant's roots, stems, and leaves. Then, PGPs of endophytic *Bacillus* strains were investigated.

2. Materials and methods

2.1. Selection and sampling of *M. longifolia* L. plants

Wilt mint (*M. longifolia* L.) were collected from Palandöken mountain of Erzurum in East Anatolia of Turkey. Samples were selected from living, healthy plants, and six individual plants were collected at a distance of 10–20 m. The samples were kept in the sterile plastic zip bag and stored at 4 °C until further use.

2.2. Sterilization and endophytic *Bacillus* isolation from *M. longifolia L.*

Sterilization and isolation of wilt mint (*M. longifolia* L.) were done according to the Zhao et al. protocol (2012). Initially, plant samples were washed with tap water for cleaning the dirt or soil particles. Then, samples were cleaned with

distilled water and immersed in 0.01% (v/v) tween-20 for 1 min after the samples were rinsed three times with sterile water and then kept wet for about 3 min in 10% aqueous NaOCl. They were rinsed again three times with sterile water and soaked in 2.5% aqueous sodium thiosulfate solution for 10 min. Then, one more time rinsed again three times with sterile water. Immersed in 75% ethanol for 30 s (leaves) or 1–5 min (roots and stems). Rinsed three times with sterile water for the last time. And then, the samples were dried overnight in sterile Petri dishes. Finally, for the understanding sterilization of the plants, parts after sterilization with ethanol, 2 uncut pieces of root, stem, and leaves were transferred on the agar media. The absence of any growth of colonies after 3 days affirmed that sterilization was accomplished. For isolation of endophytic *Bacillus* species, 10 g of samples were initially heat-treated at 80°C to eliminate vegetative cells.

2.3. Plant beneficial traits

2.3.1. Indole acetic acid production (IAA) assay

IAA production is one of the most important natural auxins, which has a key role in plant growth. The assay was performed according to Vaikuntapu et al.'s (2014) procedure. In this procedure, bacterial isolates were incubated in LB broth medium containing 0.1% tryptophan and incubated for 72 h at 28 °C. Then, the cultures were centrifuged at 6000 g for 30 min. A total of 2 mL of the supernatant was taken and mixed with 2 drops of o-phosphoric acid and 4 mL of Salkowski solvent (50 mL of 35% of perchloric acid + 1 mL of 0.5 M $FeCl_3$ solution). In the last step of the assay, indole acetic acid production was determined with the colorimetric measurement at 570 nm.

2.3.2. Nitrogen fixation assay

Nitrogen fixation ability of isolates was analyzed by using Jensen's modified nitrogen free medium (NFM) (Jensen, 1942). The medium includes following ingredients: 20 g sucrose, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4.7H_2O$, 0.2 g NaCl, 0.1 g $CaCl_2$, 0.5 g KH_2PO_4, 0.005 g Na_2MoO_4, 18 g agar, and 1000 mL distilled water. The NFM was autoclaved at 121 °C for 15 min, and then the medium was poured into Petri dishes. Then, bacterial growth was approved as potential nitrogen-fixing bacteria.

2.3.3. Phosphate solubilization assay

Potential phosphate solubilizing bacterial strains was determined by using modified Pikovskaya's (PKV) agar medium containing 10.0 g glucose, 0.5 g $(NH_4)_2SO_4$, 5 g $Ca_3(PO_4)_2$, 0.2 g NaCl, 0.1 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 0,2 g KCl; 0.002 g $MnSO_4.H_2O$, 0.002 g $FeSO_4.7H_2O$, and 1000 ml distilled water. The medium was autoclaved at 121 °C for 15 min, and then the medium was mixed carefully and poured into Petri plates. Plates were incubated at 28±1 °C for 2–7 days, and phosphate solubilization was determined according to the halo zones around the colonies (Pikovskaya, 1948)

2.3.4. Siderophore production assay

The siderophore production for each bacterial strain was determined following Louden et al.'s (2014) procedure. Firstly, all glassware were washed with 6M HCI to remove any trace elements, and it is rinsed with ddH_2O. Then, CAS agar is prepared as three main solutions containing mixture solution, blue dye solution, and CAS agar solution. After inoculation of bacterial cultures on Chrome azurol S (CAS) agar plates, the plates were incubated at 28 °C for 2–7 days. Siderophore producing bacteria indicated an alteration in color, from blue to orange around the colonies. The bacteria with an orange halo around the Petri dishes was specified as siderophore-producing bacteria.

2.3.5. Determination of the ACC (1-Aminocyclopropane-1-Carboxylate) deaminase activity

The ACC deaminase activity of isolates was evaluated using the method described by Penrose and Glick (2003). According to the method, the isolates were transferred in LB broth medium and incubated at 28 °C for 24 h. Then, 2 mL of each bacterial culture was added to tubes and centrifuged at 8000 g for 5 min. After the centrifugation step, pellets of each sample were washed two times with Dworkin and Foster (DF) minimal salts medium. Moreover, 2 mL of DF minimal salts was added with ACC, as only nitrogen source (DF-ACC) was transferred to bacterial cultures and incubated at 28 °C for 24 h. Furthermore, a noninoculated DF-ACC medium was exploited as a control group of the experiment and blank sample for the spectrophotometric measurement. Then, cultures were centrifuged at 8000 g for 5 min. One mL of each sample was taken from the cultures and centrifuged at 8000 g for 5 min. A total of 100 µL of the supernatant was diluted with 1 mL of DF medium. 60 µL of each working solution was used in the standard ninhydrin experiment (60 µL of working solution +120 of µL ninhydrin solution). The solution was mixed well and situated in a hot water bath for 30 min at 65 °C. After heat treatment, the solution turned into a purple color. The samples were cooled at the room temperature for 20 min. When compared to the control, the opaque visibility in color can be assessed as a positive result. Finally, the colorimetric measurement was performed at 570 nm.

2.3.6. Determination of ammonia potential assay

To evaluate the isolates's ability to ammonia production activity, the isolates were transferred to peptone water (Peptone 20.0 g/L and NaCl 30.0 g/L) with constant shaking at 140 rpm for 5 days at 30 °C. After incubation, 1 mL of Nessler's reagent was added to 0.2 mL of culture supernatant. When the solution color alteration was occurred from brown to yellow, the strain was determined

as ammonia production positive. Moreover, colorimetric measurement was carried out at 450 nm (Orhan, 2016).

2.3.7. HCN production assay

Production of HCN was assessed as described by the modified method of Bakker and Schippers (1987). The isolates were streaked on agar plates supplemented with or without 4.4 g glycine L^{-1}. Then, filter paper soaked in 0.5% picric acid in 1% Na_2CO_3 in the plates. After, paraffin was utilized to cover the plates and incubated at 28± 1 °C. At the final stage, color alteration on the plates from yellow to light brown, moderate brown, or strong brown was determined as HCN production.

2.4. Molecular identification of potential endophytic *Bacillus* strains

Genomic DNA isolation of endophytic bacterial isolates was extracted using the modified protocol described by Wilson (1997). Extracted DNA samples were prepared as templates for 16S rRNA gene analysis. The 16S rRNA partially gene regions were amplified using polymerase chain reaction for molecular identification of the endophytic bacterial isolates. According to this reaction, 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') were used as forward and reverse primers, respectively. The reaction was accomplished in a 30 µL reaction mixture containing 1.5 mM $MgCl_2$, 1.2 µL of dimethyl sulfoxide (DMSO), 0.2 mM each dNTP, 25 pmole of forward primer and reverse primer, 50 ng DNA template, and 5 U Taq DNA polymerase along with reaction buffer. The reaction was carried out with an initial step at 95 °C for 2 min, and 36 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C, followed by a final 5 min extension step at 72 °C, then brought down to 4 °C. Amplified 16S rRNA gene PCR products were determined by using QIAxcel advanced analysis system and sequenced by Macrogen Inc. BLAST Netherlands). The nucleotide BLAST (Basic Local Alignment Search Tool) search program of NCBI was used to determine the nucleotide sequence homology and relation to other bacterial sequences presence in the GenBank (http://blast.ncbi.nlm.nih.gov./blast.cgi). The 16S rRNA gene sequences of the endophytic bacterial strains of *M. longifolia* L. were deposited into GenBank under the accession numbers: MW564002– MW564013. Evolutionary analyses were conducted in MEGA 7.

3. Results and discussion

Sixty-five bacteria (ML1-ML65) were isolated from the root, stem, and leaves of six individual healthy plants (*M. longifolia* L.) at a distance of 10–20 m from Palandöken mountain Erzurum-Turkey in September 2020. The isolates were prepared for analyses of plant growth-promoting properties such as nitrogen fixation, phosphate solubilization, IAA, siderophore, ACC deaminase, HCN production, and ammonia potential. All the results were reported as three independent replicate experiments. All isolated endophytic strains were grown on nutrient agar (NA) then transferred to specific mediums for screening their PGPs. Also, the data of biochemical and morphological tests of potential endophytic isolates were analyzed as gram positive and bacilli shape. Moreover, as a sequence analysis method, partially 16S rRNA was used to support these results obtained by conventional methods. The isolates were 99%–100% identical to the closest relatives assigned to GenBank. Determination of nucleotide sequence homology was established via nucleotide BLAST search program of NCBI. The 16S rRNA sequences were assigned to GenBank with provided accession numbers from MW564002 to MW564013, respectively. Related information was given in Table 1, and also neighbor-joining phylogenetic tree on the basis of the partial 16S rRNA gene sequence data of the endophytic potential PGPR strains was constructed with type strains (Figure). The type strains of *Bacillus* species were chosen from among 20 clades of *Bacillus*. The type strains of *Bacillus* species were selected from among 20 different clades of *Bacillus* previously included in the literature (Gupta et al., 2020; Patel and Gupta, 2020).

The sequence results demonstrated that all active strains were grouped in *Bacillus* genera. As mentioned in Table 1, 3 *Bacillus* sp. (ML7, ML17, and ML46), 3 *B. simplex* (ML12, ML25, and ML43), 1 *B. megaterium* (ML36), 1 *B. muralis* (ML49), 1 *B. aryabhattai* (ML55), 1 *B. pumilus* (ML59), 1 *B. megaterium* (ML61), and 1 *B. toyonensis* (ML63) were isolated from roots, leaves, and stems of *M. longifolia* L. The obtained *Bacillus* strains were shown multiple PGP activities. The percentage of PGP activity of active positive isolates varies widely. Among the 12 *Bacillus* isolates, 11 *Bacillus* isolates (91.66%) showed nitrogen fixation. They contain 2 *Bacillus* sp. (ML7 and ML17), 3 *B. simplex* (ML12, ML25, and ML43), 1 *B. megaterium* (ML36), 1 *B. muralis* (ML49), 1 *B. aryabhattai* (ML55), 1 *B. pumilus* (ML59), 1 *B. megaterium* (ML61) and 1 *B. toyonensis* (ML63) were demonstrated a positive result of grown in NFM medium. Approximately, 58% of bacterial strains were determined as phosphate solubilizing activity according to the PVK media because of their halo zones. These endophytic strains are *Bacillus* sp. (ML7 and ML17), *B. simplex* (ML25), *B. muralis* (ML49), *B. aryabhattai* (ML55), *B. megaterium* (ML61), and *B. toyonensis* (ML63). IAA production potential was found about 75% of the endophytic bacterial strains containing *B. simplex* (ML12), *B. megaterium* (ML36), *B. simplex* (ML43), *Bacillus* sp. (ML46), *B. muralis* (ML49), *B. aryabhattai* (ML55), *B. pumilus* (ML59), *B. megaterium* (ML61), and *B. toyonensis* (ML63). Approximately 83% of the endophytic bacterial isolates demonstrated siderophore production. They

Table 1. Sequence similarities of endophytic bacteria isolated from the root, stem, and leaves of *M. longifolia* with sequences registered in GenBank.

Sequences of Isolated Strains Deposited to GenBank			Closest Match Among Bacteria GenBank		
Strain Names	Length (bp)	Accession Number	Reference Strain	Accession Number	Percent Identity
ML7	1391	MW564002	*Bacillus* sp.	MN160568.1	100
ML12	1415	MW564003	*Bacillus simplex*	MF177860.1	100
ML17	1200	MW564004	*Bacillus* sp.	MT598008.1	99.83
ML25	1422	MW564005	*Bacillus simplex*	MF077124.1	99.86
ML36	1465	MW564006	*Bacillus megaterium*	MT279338.1	100
ML43	1381	MW564007	*Bacillus simplex*	MT078675.1	100
ML46	1388	MW564008	*Bacillus* sp.	MW564008.1	100
ML49	1418	MW564009	*Bacillus muralis*	MG011568.1	99.86
ML55	1426	MW564010	*Bacillus aryabhattai*	MK277457.1	99.93
ML59	1381	MW564011	*Bacillus pumilus*	CP054310.1	100
ML61	1396	MW564012	*Bacillus megaterium*	MT487648.1	100
ML63	1378	MW564013	*Bacillus toyonensis*	MN173490.1	100

contain ML7, ML12, ML17, ML25, ML36, ML43, ML46, ML49, ML55, and ML59 isolates. Approximately, 42% of endophytic strains had ACC deaminase activity. These strains were *Bacillus* (ML7, ML9, ML10, ML11, ML12), and all the strains demonstrated ammonia production, but none of the *Bacillus* isolates exhibited hydrogen cyanide production. The detailed information was shown in (Table 2). According to the result of data, none of the obtained isolates showed all PGP activities.

Many extensive research has been reported beneficial effects of rhizospheric and endophytic PGP strains because of their eco-friendly, inexpensive, and sustainable usage in agricultural areas and positive effects on product yield, plant health, and development. PGPR are microorganisms, which have a beneficial relationship with the plant roots and promote plant growth via distinct PGP substances; they are also used as biofertilizers, biostimulants, and bioprotectants (Egamberdieva et al., 2015; Alaylar et al., 2018; Alaylar et al., 2019; Alaylar et al., 2020a; Alaylar et al., 2020b). Today, the world faces new diseases and, therefore, requirements of the medicines are increasing day by day. A natural compound-based medicine obtained by medicinal plants is the most preferred drug for treatments of diseases instead of chemical-based drugs, as their numerous natural compounds are extracted from medicinal plants (Toussaint et al., 2008; Nema et al., 2013). Especially, PGPRs possess various interactions with different species of host medicinal plants, commonly rhizospheric and endophytic. Rhizospheric microorganisms are generally located in the surface of the roots. Endophytics are grow within the host plants in the apoplastic space such as stems, leaves, roots, fruits, etc. (Dong et al., 1997; McCully, 2001). Especially, research on endophytic microorganism from medicinal plants and their sustainable, eco-friendly practices have been dramatically increasing worldwide day by day because of their biostimulant, biofertilizer, and biocontrol agents. *Azospirillum*, *Azoarcus*, *Arthrobacter*, *Bacillus*, *Enterobacter*, *Azotobacter*, *Clostridium*, *Gluconacetobacter*, *Pseudomonas*, *Serratia* are the well-known endophytic genera isolated from a distinct type of medicinal plants (Alaylar et al., 2018; Alaylar et al., 2019; Alaylar et al., 2020a). Among these genera, the genus *Bacillus* has been demonstrated as one of the highest potential PGPR strains, which have biocontrol, biofertilizer, and biostimulant properties according to the literature (Borriss, 2011; Kaki et al., 2013). Research on beneficial endophytic microorganisms from medicinal plants and their application in sustainable agricultural practices have increased all over the world, and they have been reported as plant growth-promoting bacteria and biocontrol agents. There are a few similar results that have reported various aromatic and medicinal plants. For instance, Huang et al. (2019) isolated *Bacillus* sp. and *B. aryabhattai* from *Platycodon grandiflorum* that have nitrogen-fixing capability. Another study was conducted by Karthikeyan et al. (2010). They isolated some PGPR strains from *Catharanthus roseus,* and, among these strains, *B. megaterium* has increased the nutrient uptake. Murugappan et al. (2013) noted that *B. pumilus* were isolated from the stem and leaves of *Ocimum sanctum*. According to their data, *B. pumilus* showed most of the PGPs such as IAA production, phosphate solubilization, ammonia, HCN, and siderophore production. Further, Ahmed et al. (2014) evaluated *Bacillus* sp. from several

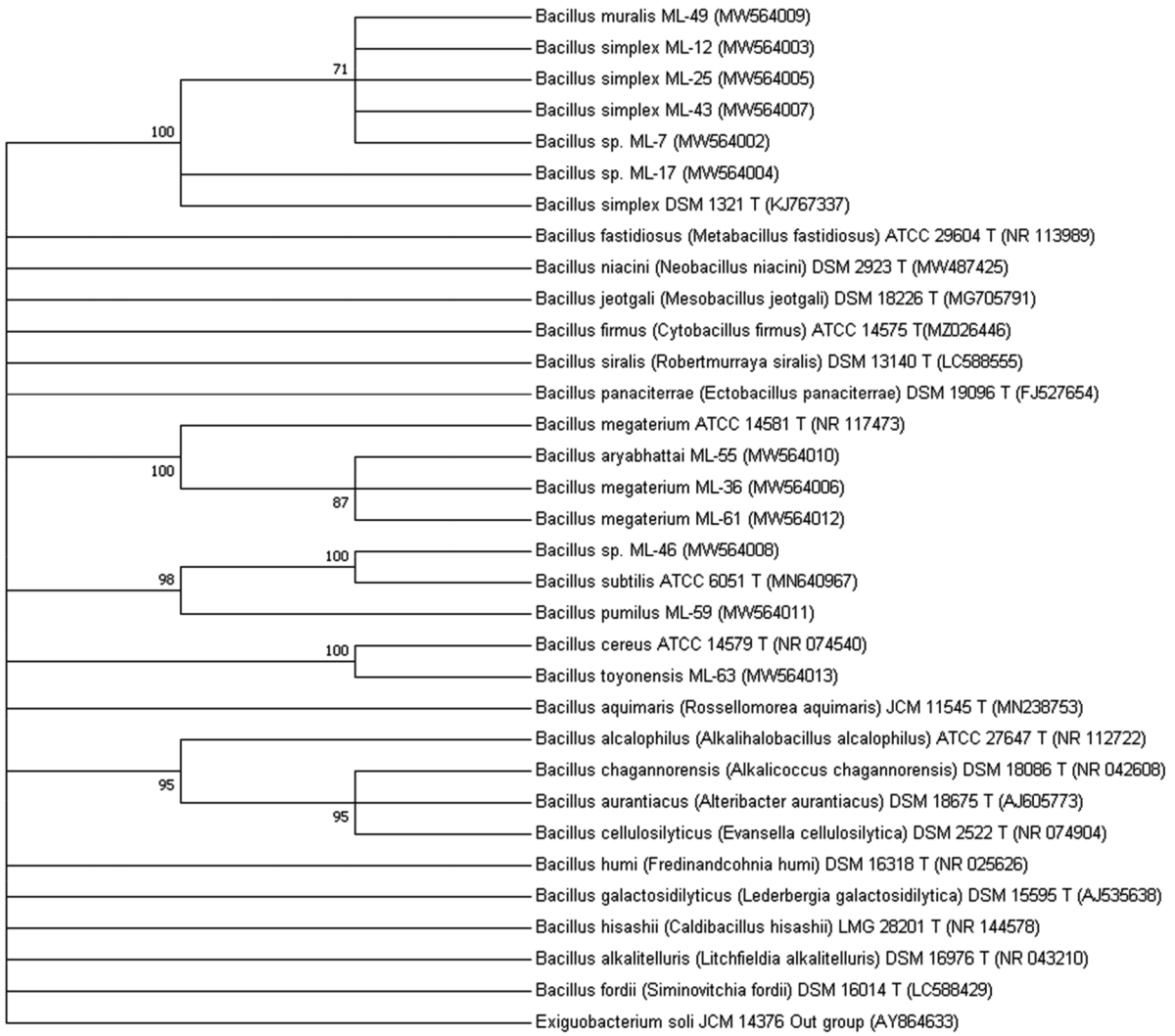

Figure. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences strains from endophytic *Bacillus* strains of *M. longifolia*, showing the relationship of isolated strains to their nearest relatives in GenBank. Branches corresponding to partitions reproduced in less than 70% bootstrap replicates are collapsed.

medicinal plants for plant growth promotion and biological control in Andhra Pradesh-India. A study by Nnzeru et al. (2017) demonstrated that *Bacillus* strains from numerous medicinal plants in South Africa and the *Bacillus* reported the most dominant endophytic genus. Ansary and coworkers (2018) isolated plant growth-promoting endophytic bacteria under *Bacillus* genus from traditional herbal medicinal plants such as *Duranta plumeri*, *Ocimum gratissimum* L., *Terminalia bohera* and *Manihot esculenta* in Dhaka of Bangladesh, and these *Bacillus* strains were demonstrated high IAA production. Li et al. (2008) isolated endophytic bacteria from *Glycyrrhiza uralensis*. They have reported *Bacillus* sp. that can be used as promising isolates for biofertilizers to promote improved survival and growth of licorice and other valuable crops in arid lands in Xinjiang province of China. Sahu et al. (2020) have also reported eight endophytic *Bacillus* isolates from *Ocimum tenuiflorum* L., which have biocontrol potential because of their PGP activity. The results are obtained with previous reports, Vendan et al. (2010) and Akinsanya et al. (2015) revealed *Bacillus* in all tissues in *Aloe vera*. Moreover, *Bacillus* species were reported to situate as endophytes of medicinal plants such as *Lonicera japonica*, *Rumex pulcher,* and *Tridax procumbens,* etc. (Tamilarasi et al., 2008; Ebrahimia et al., 2010; El-Deeb et al., 2013; Preveena and Bhore, 2013; Ahmed et al., 2014; Akinsanya et al., 2015).

The result of the present study carried out that all endophytic *Bacillus* species possess multiple PGP activity such as ACC deaminase activity, nitrogen fixation,

Table 2. Determination of plant growth promoting properties of endophytic *Bacillus* strains.

Strains Names	N_2 fixation	Phosphate solubilization	IAA production	ACC-deaminase production	Siderophore production	Ammonia production	HCN production
ML7	+	+	–	–	+	+	–
ML12	+	–	+	–	+	+	–
ML17	+	+	–	–	+	+	–
ML25	+	+	–	–	+	+	–
ML36	+	–	+	–	+	+	–
ML43	+	–	+	-	+	+	–
ML46	–	–	+	+	+	+	–
ML49	+	+	+	–	+	+	–
ML55	+	+	+	+	+	+	–
ML59	+	–	+	+	+	+	–
ML61	+	+	+	+	–	+	–
ML63	+	+	+	+	–	+	–

"+" positive; "–" negative".

phosphate solubilization, siderophore, IAA, ammonia, and HCN production. A total of 12 *Bacillus* isolates were identified as potential endophytic PGP strains, which have been isolated from *M. longifolia* L. According to the previous studies, there is significant information about endophytic *Bacillus* strains, which have a key role in medicinal plants with their biocontrol, biostimulant, and bioprotectan properties. Therefore, *Bacillus* species can be used in plant growth, nutrient uptake and diminish the presence of plant diseases. Hence, increasing the soil fertility and quality by innovative technologies are essential for agricultural demands. Also, biofertilizers, biostimulants, and bioprotectans have been utilized worldwide instead of chemical fertilizers for food security and sustainability. To the best of our knowledge, this is the first study searching of potential endophytic *Bacillus* strains, which have isolated and reported their PGP activity from *M. longifolia* L. in Palandöken mountains Erzurum, Turkey. Our results suggested that endophytic microorganisms have a huge potential of cultivable endophytic bacteria exhibiting multiple PGP activities. Therefore, beneficial metabolites of endophytic microorganisms, which are obtained from medicinal plants, can be used in food and medicine industry to struggle with plant pathogens, nutrient uptake of plants. It is also noted that food-based plants are immense sources of PGP, and medicinal plants are promising alternative sources with natural compounds of endophytic bacteria.

4. Conclusion

This study illustrates that endophytic *Bacillus* strains isolated from a medicinal plant (*M. longifolia* L.) in Palandöken Mountain Erzurum-Turkey. Totally, 12 *Bacillus* species were isolated and identified. These strains have possessed multiple PGP activities, so that this let native local potential endophytic PGP strains be used as biofertilizers, biositimulants, and bioprotectants because of their multiple PGP activities. Hence, these strains can be used as an effective approach instead of chemical fertilizers to improve plant growth, nutrient uptake, suppress pathogens, etc., for medicinal plants in sustainable agricultural applications.

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