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RESEARCH ARTICLE

Isolation and characterization of endophytic bacteria from *Plectranthus tenuiflorus* medicinal plant in Saudi Arabia desert and their antimicrobial activities

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The diversity and beneficial characteristics of endophytic microorganisms have been studied in *Plectranthus tenuiflorus* medicinal plant. However, information regarding naturally occurring *P. tenuiflorus* plant associated endophytes among different organs of host is limited. Endophytic bacteria were isolated from root, stem, and leaves of *P. tenuiflorus* plant. Among 28 endophytic bacterial isolates from different organs of *P. tenuiflorus* plant, 8 isolates were identified by partial sequencing of their 16S rRNA gene. The isolated endophytic bacteria were *Bacillus* sp., *Bacillus megaterium, Bacillus pumilus*, *Bacillus licheniformis*, *Micrococcus luteus*, *Paenibacillus* sp., *Pseudomonas* sp., and *Acinetobacter calcoaceticus*. The most isolates that exhibited extracellular enzymatic activity were belonged to the genus *Bacillus*. Furthermore, *Bacillus* sp. (HE613660) exhibited the stronger activities in extracellular enzymes such as amylase, esterase, lipase, protease, pectinase, xylanase, and cellulase than other strains. Considerable antimicrobial activities against a panel of human pathogenic microorganisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae, Proteus mirabilis,* and *Candida albicans*) were recorded using crude extracts of the collected endophytic strains.

Keywords: *Plectranthus tenuiflorus*; endophytic bacteria; antimicrobial substances; extracellular enzymes

Introduction

Endophytic bacteria live in plant tissues without doing substantive harm or gaining benefit other than residency (Kobayashi and Palumbo 2000). Hallmann et al. (1997) defined an endophyte as any micro-organism that resides inside the plant without regard to the specific tissue colonized and these bacterial endophytes can be isolated from surface-disinfected plant tissue or extracted from internal plant tissue. Endophytic bacteria seem to be distributed in most plant species and have been isolated from roots, leaves, and stems, and a few from flowers, fruits, and seeds (Lodewyckx et al. 2002). Endophytic bacteria may accompaniment certain metabolic properties, such as promoting plant growth, controlling soil-borne pathogens, or helping host plant to defeat stress responses to environmental abuse (Mastretta et al. 2006; Taghavi et al. 2007; Ryan et al. 2008). Furthermore, the interactions between plants and bacteria help plants to settle in ecosystem restoration processes (Glick et al. 1995). These interactions may increase the ability of plants to utilize nutrients from the soil by increasing root development, nitrate uptake or solubilizing phosphorus, and to control soil-borne pathogens (Whipps 2001).

Plant communities in arid habitat are controlled by the interaction between biotic and physico-chemical components of the desert matrix (Read 1998). Interactions with microbes appear crucial in obtaining inorganic nutrients or growth-influencing substances. Generally, plant growing in unique environmental settings having special ethnobotanical uses having extreme age or interesting endemic locations possess novel endophytic micro-organisms which can supply new leads. About 51% of biologically active substances isolated from endophytic fungi were previously unknown (Strobel 2003). Recently, many known as well as new endophytic bioactive metabolites, possessing a wide variety of biological activities as antibiotic, antiviral, anticancer, anti-inflammatory, antioxidant, etc., have been identified (Strobel and Daisy 2003). Endophytes existing in plants have a wide range of antimicrobial strains, which are the important potential sources of antimicrobial substances (Strobel 2003). Some endophytes could excrete antimicrobial compounds that may be involved in a symbiotic association with a host plant (Yang et al. 1994; Strobel 2003). Many biologically active substances that endophytes excrete were relatively new to us (Schulz et al. 1995). In addition, the antibiotics made by endophytes may reduce cell toxicity toward higher organisms because the plant itself serves as a natural selection system (Strobel 2003). Therefore, it is a huge potential to screen novel, highly active, and low toxicity antimicrobial substances from endophytes.

Plectranthus is a genus of about 300 species (Miller et al. 1988; Abdel-Mogib et al. 2002; Lukhoba

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et al. 2006) that are traditionally used to treat skin, digestive, and respiratory diseases (Alsabahi et al. 1999; Lukhoba et al. 2006). *Plectranthus tenuiflorus* (*Euphorbiaceae* family) is one of the medical important species of the genus *Plectranthus* that grows naturally in the Taif province in high-altitude mountains Southwest of Saudi Arabia (Mossa et al. 2000; Rahman et al. 2004). In Tiaf, *P. tenuiflorus* is known as 'Shara.' It grows in dry areas to about 50 cm in height with perennial succulent leaves that are opposite and an inflorescence consisting of cylindrical spike of flowers. The plant has a pleasant aroma similar to the commercial product 'Vicks.' In Taif, it is used as an eardrop for earache and inflammation of middle ear (Chandrasekaran and Venkatesalu 2004), whereas it is prescribed in Asia for a reedy sore throat (Rahman et al. 2004). Few studies have been conducted on the chemotype and activity of the essential oils from different species of *Plectranthus*. The oil of *Plectranthus barbatus* is antiallergic (Gupta et al. 1993); whereas, the oil of *Plectranthus incanus* is active against *Staphylococcus aureus* and that of *Plectranthus glandulosus* is active against *Escherichia coli* and *S. aureus* (Varma and Sharma 1963; Jirovetz et al. 2002), respectively. Various investigators reported endophytic microbes from various plant exists in different ecosystems. Consequently, the opportunity to find new and interesting micro-organism among myriads of plants in different settings and ecosystems is great. Previously, numerous reports showed on diversity of endophytic bacteria, fungi in medicinal plants (Strobel 2003), but from available literature there is no report on endophytic bacteria from *P. tenuiflorus* particularly in Saudi Arabia desert. Therefore, the present study aimed to study endophytes associated with medicinal plants used as ethno medicine by the tribal communities of Taif province, Saudi Arabia and to evaluate these endophytes for antimicrobial activity against some human pathogens. This study also focuses on obtaining a comprehensive picture of the endophytic bacterial community of the *P. tenuiflorus* plant growing on an arid habitat

Materials and methods

Micro-organisms, plasmids and media

Putative endophytic bacteria strains were isolated from *P. tenuiflorus* and cultured at 28 or 37°C in tryptic soy agar (TSA) medium containing of 15 g Trypticase peptone, 5 g phytone peptone, 5 g NaCl, 15g Agar per liter of water and/or (PDA) medium comprising of 200 g and potato infusion, 20 g dextrose, and 15 g Agar. The pH of the medium was adjusted to 7.2–7.4. Medium A (10 g polypeptone, 10 g glucose, 1 g KH_2PO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per 1l, pH 6.8) was used for antibiotic production.

Surface sterilization of plant samples

Plectranthus tenuiflorus plant was collected during summer from Al hada and Al shafa (Lat. 99°44′ and 79°45′E), Taif province, Northwest of Saudi Arabia were brought to the laboratory in sterilized containers. Plant organs were washed in running tap water and graded by size and surface appearance in order to exclude samples that showed symptoms of disease or superficial damage. Endophytic bacteria were isolated from stem, leaves, and root regions of selected plants. The plant parts were washed thoroughly in running tap water and surface sterilized with sodium hypochlorite (2%) containing 0.1% Tween 20 for 3 min. for stems, leaves, and roots. The disinfectant was removed by rinsing five times each in two washes of sterile distilled water and finally in sterile water and plant parts were dried on sterile paper towels (Hallmann et al. 1997; Zinniel et al. 2003) dissected into 1 cm pieces and then pressed onto nutrient agar as a disinfestations control. To confirm that the disinfection process was successful, the plant organs pieces were pressed onto TSA medium plates and aliquots of the sterile distilled water used in the final rinse were also plated onto the same medium. The plates were examined for growth after incubation at 28°C for 3 days.

Isolation of endophytic bacteria

The surface-disinfected plant organs (roots, stems, and leaves) were removed with a sterilized razor blade, and the organs were cut into pieces 1–3 mm-long, which were placed on TSA plates. Incubation was carried out at 28°C for 1–7 days to allow growth of endophytic bacteria. In a further experiment, fragments of roots, stems, and/or leaves were macerated in 5 mL of 12.5 mM potassium phosphate buffer (pH 7.0) with sterile mortar and pestle. Tissue extracts were then serially diluted in potassium phosphate buffer (pH 7.0) and plated in triplicate to recover any bacterial endophytes present in the plant tissue. The plates were incubated at 28°C for 1–7 days or until growth was observed, upon which the CFU were counted and the population density was estimated. Following incubation, bacteria recovered from each plant organs pieces and/or homogenized sample were selected at random, purified, and grouped on the basis of phenotypic characteristics, e.g., colony morphology, colony color, cell shape, motility, growth rate, and Gram reaction. Ten isolates representing each bacterial group of interest were selected for further identification

Phenotypic and genotypic characterization of selected isolates

Standard tests were performed for identification of the studied strains in accordance with *Bergey's Manual of Determinative Bacteriology* (1994). Cell form and size, gram staining, spore formation,

motility, colony pigmentation, and production of UV-fluorescent pigments were studied. The strains were also identified by 16S rRNA gene sequences. The genomic DNA of endophytic bacteria was extracted (Govindarajan et al. 2007), and 16S rDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and bacterial universal primers, 27 F (5′-GAGTTTGAT CACTGGCTCAG-3′) and 1492 R (5′-TACGGC TACCTTGTTACGACTT-3′) (Byers et al. 1998). Briefly, a 25-µl reaction mixture contained 1.25 U Taq polymerase (Sigma Chemical Co., St. Louis, MO, USA.), 0.2 mM dNTPs, 25 mM $MgCl_2$ (Sigma), 10 pmol of each primer, 2.5 µL of 10 × reaction buffer (Sigma), and 1 µg of template DNA. Aliquots of PCR reaction products were electrophoresed in 1% agarose, containing 10 µg mL^{-1} ethidium bromide. To know the identity of organism, obtained sequences were compared with nucleotides databases like GenBank (Benson et al. 2009).

Exoenzyme activity tests

The agar diffusion method was used to detect extracellular hydrolytic enzyme activity. The isolates were grown on different indicator media including cellulase activity indicator medium (LB medium with 0.5% (w/v) carboxylmethylcellulose and 1.5% agar (w/v) (Farkas et al. 1985), xylanase activity indicator medium (LB medium containing 0.5% (w/v) oat spelt xylan and 1.5% agar (w/v) (Farkas et al. 1985), amylase activity indicator medium (LB medium containing 1.0% (w/v) starch and 1.5% agar (w/v) (Claus 1988), protease activity indicator medium (LB medium containing 1.0% (w/v) skim milk and 1.5% agar (w/v) Claus (1988), lipase activity indicator medium (LB medium containing 1.0% tricaprylin (v/v) and 1.5% agar (w/v) (Lusty and Doudoroff 1966), esterase activity indicator medium (LB medium containing 1.0% tributyrin (v/v) and 1.5% agar (w/v) (Lusty and Doudoroff 1966), pectinase activity indicator medium (LB medium containing 0.5% polygalaturonate (v/v) and 1.5% agar (w/v) (Collmer et al. 1988). Bacterial cultures were incubated at 30°C for 48 h. A clearing zones in the medium indicated positive enzyme activity were recorded.

Evaluation of antimicrobial activity

Six common antibiotic resistant human pathogens such as *Salmonella typhi* (ATCC-51812) *S. aureus* (ATCC 29213), *E. coli* (ATCC 9637), *Klebsiella pneumoniae* (ATCC 37853), *Streptococcus agalactiae*, *Proteus mirabilis*, and fungal pathogen *Candida albicans* (ATCC 10231) were used to evaluate the antimicrobial activity of endophytic crude extracts. The bacterial strain was inoculated into medium A broth (100 mL) in a 250-mL Erlenmeyer flask and incubated in a rotary shaker (150 rpm) at 30°C for 48 h. Cell-free supernatant was obtained by centrifugation (10,000 × *g*, 15 min) and filtered on a 0.45 lm Millipore filter. The antimicrobial activity of the culture filtrate was detected by the method of stainless steel cylinders (Yoshida et al. 2001). The suspension of each indicator strain (0.5 mL) was mixed with 10 mL of suitable agar media at 55°C, and the mixture was immediately poured in a 9 cm diameter petri dish. After solidification, sterilized stainless cylinders (6 mm internal diameter and 10 mm high) were equidistantly placed open end up on each plate. Filter sterilized culture filtrates (200 µL) of each bacterial strain were added to the cylinder. After incubation under suitable conditions and time, the diameter of a round inhibition zone against the indicator strains was measured with calipers.

Results and discussion

Frequency, diversity, and identity of endophytic bacteria from Plectranthus tenuiflorus plant

The endophytic bacterial communities of healthy looking roots, stems, and leaves of the medicinal plants (*P. tenuiflorus*) were assessed in surface disinfested plant parts upon cultivation in TSA and PDA medium. The result of effectiveness of the surface sterilization protocol was determined. Rinsed water of each sample showed no microbial growth on TSA medium after incubation at 30°C for 15 days, indicating that the epiphytic microbes were completely removed by this surface sterilization procedures. Indeed, the major key to succeed in isolating and studying endophytes is to ensure the sterility of the plant surface (Hallmann et al. 1997). The diversity of isolated endophytic bacteria was also largely dependent on the isolation methods (Das et al. 2007). The number of colony-forming units per gram fresh weight (CFU) of culturable endophytic bacteria isolated from various plant organs of *P. tenuiflorus* plants was determined (Table 1). The results showed that CFU value in roots (1.5×10^2) is much lower than in stems (2.4×10^3) of *P. tenuiflorus* plants. In contrast, leaves had higher CFU values than other organs ($\sim 2.9 \times 10^4$). This results are in agreement with the results obtained by Jalgaonwala et al. (2010)

Table 1. Endophytic bacterial population recovered from different organs of *Plectranthus tenuiflorus* (cfu g^{-1} FW).

	TSA medium			PDA medium		
Host plant	Root	Stem	Leaves	Root	Stem	Leaves
Plectranthus tenuiflorus	1.5×10^2	2.4×10^3	2.9×10^4	1.1×10^2	2.1×10^3	2.2×10^4

whom demonstrated that population density of endophytes in medicinal plant seems to be highest in aerial tissues than the underground tissues. In addition, the population densities reported in the present study are less than the earlier reports in crops such as sugar beet (10^3–10^5 CFU./g FW of roots) (Bugbee et al. 1975) and potato (3.3×10^5 CFU/g fw of stem) (De Boer and Copeman 1974). This may be attributed to the desert habitat of *P. tenuiflorus* plants. Previous reports demonstrated that the population of endophytic bacteria in wild plant growing in desert was lower than average levels of endophytes in crop plants (El-Deeb et al. 2011; Lopez et al. 2011). Furthermore, our results indicated that TSA supported more of endophytic bacterial growth than PDA (Table 1) as previously reported (Hung and Annapurna 2004; El-Deeb et al. 2011).

Phenotypic and genotypic characterization of endophytic bacteria

Based on the visible morphological differences, totally 28 bacterial strains were isolated from *P. tenuiflorus* plant samples. According to *Bergey's Manual of Determinative Bacteriology* (1994) the collected strains belonged to different genera (*Bacillus*, *Paenibacillus*, *Micrococcus*, *Pseudomonas*, and *Acinetobacter*). Representative isolates were chosen from each plant organ for further analysis (Table 1). For accurate identification 16rRNA Sequence analysis was performed by using the algorithms BLAST (National Center for Biotechnology Information [http://www.ncbi.nlm.nih.gov]. Multiple-sequence alignment methods were conducted using a freely available alignment program, Clustal X (version 1.81). Bacterial identifications were based on 16S rRNA gene sequence similarity (Halda-Alija, 2003, 2004). Neighbors joining phylogenetic tree (Saitou and Nei 1987) were generated using sequence data from gene bank for strains that showed high percentage of similarities with our strains (Figure 1). The Phylogentic tree indicated that each bacterial strains from *P. tenuiflorus* clustered to its corresponding strain from gen bank with 100% boot strap factor. The 16S rRNAsequences of endophytic bacteria reported in this article have been deposited in the GenBank database under accession numbers: HE613660 (*Bacillus* sp), HE613653 (*Bacillus pumilus*), HE613654 (*Paenibacillus* sp), HE613655 (*Bacillus megaterium*), HE613657 (*Bacillus licheniformis*), HE613658 (*Micrococcus luteus*), HE613660 (*Pseudomonas* sp.), and HE613656 (*Acinetobacter calcoaceticus*).

Previously numerous reports studied diversity of endophytic bacteria, and fungi in medicinal plants (Jalgaonwala et al. 2010). The endophytic bacterial community isolated from *P. tenuiflorus* included *Paenibacillus* sp., *B. megaterium*, and *Pseudomonas* sp. has been previously characterized as a Korean ginseng root endophytes (Cho et al. 2007). *Paenibacillus* has also been found as an endophyte in different woody plants like pine, coffee, and poplar (Sakiyama et al. 2001; Bent and Chanway 2002). *Acinetobacter, Bacillus, Pseudomonas* have been identified as an endphytes in *Echinacea* medicinal plant (Lata et al. 2006), while *B. pumilus, Bacillus subtilis, B. megaterium, Pseudomonas mendocina* were isolated as an Endophyte from the root of Medicinal Plant *Chlorophytum borivilianum* Safed musli (Panchal and Ingle 2011). *B. licheniformis* has been identified in *Jacaranda decurrens* plant (Carrim et al. 2006).

Evaluation of hydrolytic enzyme activities

The endophytic bacteria isolated from the *P. tenuiflorus* were evaluated for the presence of active hydrolytic enzymes including cellulase, xylanase, pectinase, amylase, protease, lipase, and esterase (Table 2). Among the isolated bacterial strains, especially *Bacillus* sp. *B. pumilus*, *B. licheniformis*, *B. megaterium*, and *Paenibacillus* sp. showed maximum number of enzymes activities among seven tested enzymes activities. The amylolytic activity was observed for isolates *Bacillus* sp., *B. licheniformis*, *B. megaterium*, *B. pumilus*, and *Pseudomonas* sp. (Table 2). Previous reports demonstrated that *Pseudomonas stutzeri*, *B. megaterium*, and *B. licheniformis* have been suggested as producers of extracellular amylase (Schmidt and John 1979; Rivera et al. 2003). The Lipolytic activity was only observed for isolates *B. pumilus*, *B. megaterium*, and *Pseudomonas* sp. (Table 2). These strains have been suggested as producers of extracellular lipase (Jung et al. 2003; Ruiz et al. 2002). Protease producer isolates were member of *Bacillus, Paenibacillus*, and *Pseudomonas* (Table 2). The presence of proteolytic activity in the genus *Bacillus* confirmed that some species of *Bacillus* tended to synthesize proteolytic enzymes during the sporulation process (Ponnuraj et al. 1999; Kamasaka et al. 2002). *Paenbacillus* species are known to produce different hydrolyzing enzymes (Sakiyama et al. 2001). Generally, in presence study, Gram-positive bacteria displayed broad hydrolase potential than Gram-negative bacteria (Sanchez-Porro et al. 2003; Rhoban et al. 2009). Cho et al. (2008) isolated two cellulose hydrolase genes (cel5A and cel5B) from endophytic *Paenibacillus polymyxa* GS01 of ginseng roots. In general, the hydrolytic enzymes of endophytes appear to be important for the colonization of plant roots (Quadt-Hallmann et al. 1997; Reinhold-Hurek and Hurek 1998; Sakiyama et al. 2001). This hypothesis is supported by the presence of cellulolytic and pectinolytic enzymes produced by numerous endophytic bacteria such as *Rhizobium* sp. (Al-Mallah et al. 1987). Verma et al. (2001) demonstrated the presence of varying levels of cellulase and pectinase activities in different isolates, possibly affecting their potential for inter/intracellular colonization. In addition, bacteria enter

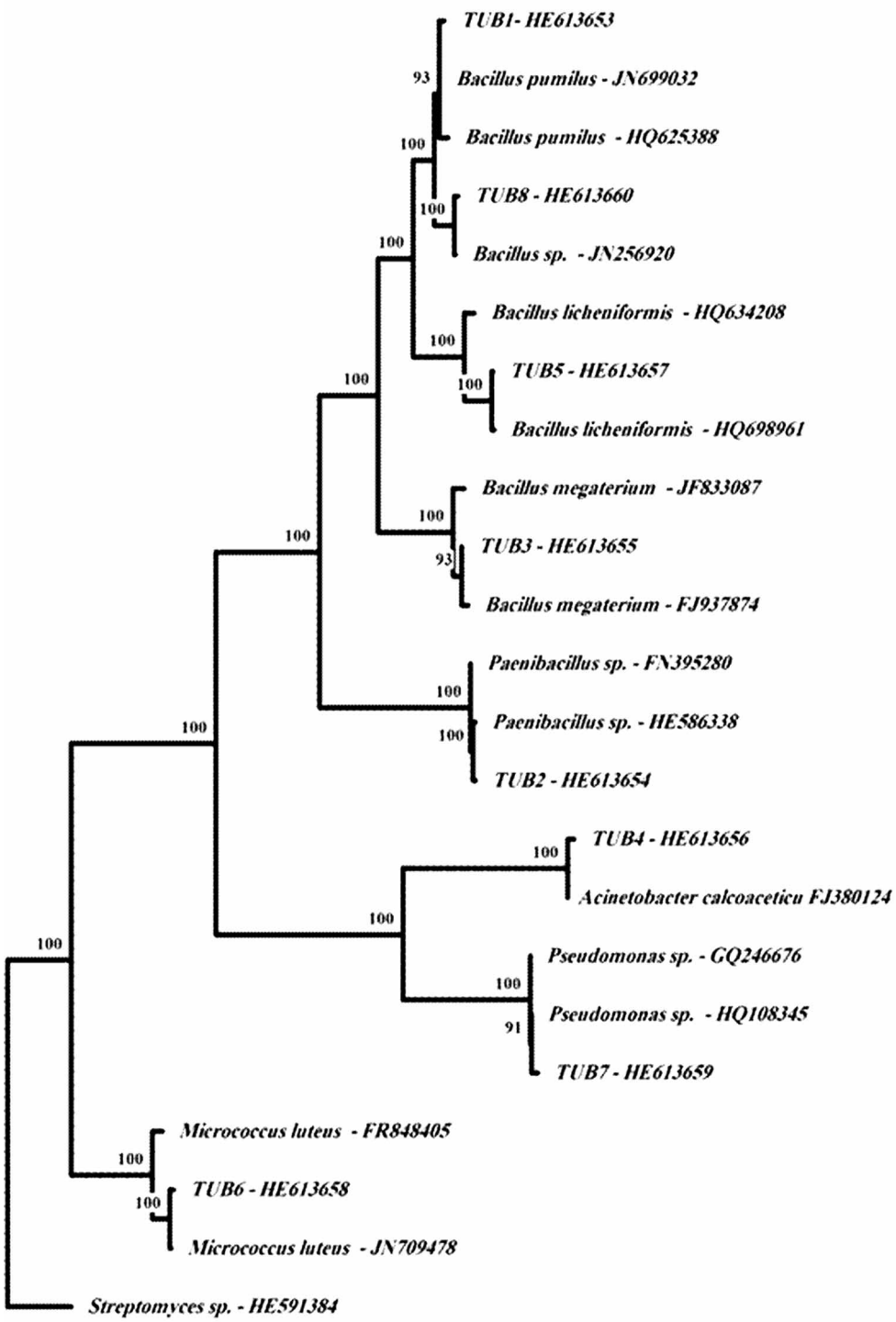

Figure 1. Neighbor-joining tree based on sequence data from 16s rRNA gene. The value on each branch is the percentage of bootstrap replications supporting the branch.

the interior of the root by hydrolyzing wall-bound cellulose, auxin-induced tumors, water flow, and wounds, or where the lateral roots branch (Al-Mallah et al. 1987). Therefore, the endophytic bacteria can be the new source for commercial enzyme production. Moreover, endophytes recognized as potential sources of novel natural products for exploitation in medicine, agriculture, and industry with more and more bioactive natural products isolated from the micro-organisms (Guo et al. 2008).

Evaluation of anti-human pathogenic activity

Bacterial endophytes have been recognized as repository of novel secondary metabolites for potential therapeutic use (Tan and Zou 2001). Further, Strobel and Daisy (2003) necessitated that medicinal and endemic plants should use for endophytic studies as they are expected to harbor rare and interesting endophytes with novel bioactive metabolites. This has lead to the discovery of several bioactive compounds from fungal and bacterial endophytes and

Table 2. Evaluation of extracellular hydrolytic enzyme activity from the endophytic bacteria isolated from *Plectranthus tenuiflorus* the medicinal plant.

Bacterial strains	Plant organ	Amylase	Esterase	Lipase	Protease	Pectinase	Xylanase	Cellulase
Bacillus sp.	Root	+++	+	−	+++	++	+++	+++
Bacillus pumilus	Leaves	++	++	++	+++	+++	++	+
Paenibacillus sp.	Leaves	++	++	−	+++	+++	+++	+++
Bacillus licheniformis		++	+	−	++	++	+++	++
Bacillus megaterium	Leaves	++	++	+++	++	+++	−	−
Micrococcus luteus	Stem	−	++	−	+	−	+	+
Acenitobacter sp.	Stem	−	+	−	−	−	−	−
Pseudomonas sp.	Root	−	++	+	+++	−	−	+++

Note: Indicate no inhibition zone; + indicate inhibition zone > 5 mm; ++ indicate inhibition zone < 5 mm; and +++ indicate inhibition zone < 8 mm.

wealth of literature on antimicrobial activity of endophytic bacteria and fungi isolated from medicinal plants (Li et al. 2006; Raviraja et al. 2006; Tayung and Jha 2006). In present study, the crude metabolites extracts of endophytic bacteria isolated from *P. tenuiflorus* plant showed considerable antimicrobial activity against a panel of human pathogenic micro-organisms (Table 3). Out of the 28 endophytic bacteria isolated from different organs of the *P. tenuiflorus* medicinal plant, 8 isolates (28.5%) could display antimicrobial activity inhibiting at least one of the test pathogens (Table 3). Furthermore, among the potent strains, 14.2% displayed both antibacterial (Gram-positive and Gram-negative) and antifungal activity (Table 3). Among the bacteria isolated from root samples, *Bacillus* sp. and *Pseudomonas* sp. displayed significant antimicrobial activity against entire test pathogens (Table 3). Endophytic bacteria with potent antibacterial activity isolated from roots of *Solanum* sp. were reported by Long et al. (2003). Furthermore, *B. megaterium* and *B. licheniformis* isolated from *P. tenuiflorus* leaves exhibited antibacterial activity against *E. coli* (ATCC 9637), *S. aureus* (ATCC 29213) and *S. typhi* (ATCC-51812). It has been reported that *Bacillus* sp., *B. licheniformis*, *Paenibacillus* sp., *B. pumilus*, and *B. subtilis* isolated from medicinal plant as endophytes produce antibiotics (Madigan et al. 2005). In addition, among the genus of *Bacillus*, only *Paenibacillus* sp. isolated from leaves and *Bacillus* sp. isolated from root samples exhibited antifungal activity against *C. albicans*. Generally, the extract of endophytic bacteria was significantly effective against both Gram-positive and Gram-negative bacteria (Table 3) and moderately effective against the fungal pathogen *Candada albcans*. Endophytic bacteria produce antibiotics, which can act against human pathogenic bacteria, have previously been reported (Seo et al. 2010). Thus, endophytes can be a good source for the industrial production of antibiotics.

Table 3. Evaluation of in vitro inhibitory activity against the human pathogenic bacteria by *Plectranthus tenuiflorus* endophytic bacteria (diameter of inhibition* (mm).

Endophytic Bacterial strains	Plant organ	*Salmonell typhi* (ATCC-51812)	*Staphylococcus aureus* (ATCC 29213)	*Escherichia coli* (ATCC 9637)	*Klebsiella pneumoniae* (ATCC 37853)	*Streptococcus agalactiae* LC	*Proteus mirabilis* LC	*Candida albicans*
Bacillus sp.	Root	15.23 ± 0.68	13.45 ± 1.01	14.12 ± 0.55	13.6 ± 1.	11.12 ± 0.23	14.23 ± 1.02	10.12 ± 0.53
Bacillus pumilus	Leave	–	–	–	-	-	13.2 ± 0.85	–
Paenibacillus sp	Leaves	12.45 ± 0.34		12.23 ± 0.64			11.23 ± 0.64	13.8 ± 0.96
Bacillus megaterium	Leaves	11.54 ± 0.45	13.2 ± 0.86	11.56 ± 0.53	–	–	–	–
Bacillus licheniformis	Leave	–	12.23 ± 0.65	12.36 ± 0.66	–	–	–	–
Micrococcus luteus	Stem	–	–	–	–	–	10.80 ± 0.75	–
Pseudomonas sp.	Root	12.23 ± 0.67	11.56 ± 0.43	12.4 ± 0.74	11.7 ± 0.38	12.45 ± 0.54	10.22 ± 0.64	10.7 ± 0.66
Acinetobacter calcoaceticus	Root	10.34 ± 0.82	–	–	–	–	10.56 ± 058.	–

Note: Crude extract activity is expressed as the size of inhibition zones: –, no inhibition. *Values are the means – standard deviations of quadruple measurement; LC, laboratory collection; ATCC, American Type Culture Collection.

Conclusion

This study evidenced that *P. tenuiflorus* is the potential but under exploited resources for bioactive endophytic bacteria since the exploited bacteria isolated from *P. tenuiflorus*, in this study, showed promising antimicrobial, and enzymatic activities. Detailed investigations on *P. tenuiflorus* plant endophytic bacteria were needed to prove its potential further and if will leads to the discovery of numerous value metabolites (current experiments in our Lab.). This aspect of antimicrobial activity will be further investigated to enhance production of secondary metabolites of interest.

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