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Diversity and antimicrobial activity of isolated endophytic bacteria from Deodeok (*Codonopsis lanceolata*) of different locations and ages

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Deodeok (*Codonopsis lanceolata*) were collected from two mountainous areas (Jirisan and Yarongsan) in Korea. Endophytic bacteria were isolated from two, five, and seven-year-old deodeok (*Codonopsis lanceolata*) roots. The bacterial diversity of deodeok root samples was evaluated by 16S rDNA analysis. In the roots collected from Jirisan, we identified the presence of *Bacillus polyfermenticus* (JR2-1), *Bacillus subtilis* (JR2-3 and JR5-5), *Bacillus licheniformis* (JR2-6), and *Bacillus pumilus* (JR5-4 and JR7-2). The various *Bacillus* genera, such as *B. polyfermenticus* (YR5-1 and YR7-4), *B. licheniformis* (YR5-2 and YR7-1), and *B. subtilis* (YR7-2), were found in the roots derived from Yarongsan. *Bacillus pumilus* (JR5-4 and JR7-2) isolated from Jirisan exhibited antifungal activity against plant pathogenic fungi. *B. subtilis* (JR5-5) and *B. licheniformis* (JR2-6 and JR7-4) showed antifungal activity against *Phytophthora capsici*, *Fusarium oxysporum*, and *Rhizoctonia solani*. This newly demonstrates the antimicrobial activity ability and bacterial diversity of deodeok roots at different ages by comparing two areas that exhibited antimicrobial activity against human pathogens.

Key words: Antimicrobial activity, bacterial diversity, deodeok roots, endophytic bacteria.

INTRODUCTION

Deodeok (*Codonopsis lanceolata*), belonging to family *Campanulaceae*, has been used as a traditional medicinal plant in Korea, Japan, and China. *C .lanceolata* is a flowering plant and is a variety of bonnet bellflower. *C. lanceolata* is called deodeok in Korea and well known for its phytomedical activities, such as antimicrobial, antioxidant, and immune-defense activities (He et al., 2011). Deodeok roots have been used as an herbal drug to treat bronchitis, cough, spasm, and inflammation, and as a tonic rude drug and are also consumed as a vegetable (Lee et al., 2002; Kim et al., 2010). Important diseases affecting deodeok roots are *Phytophthora* blight, *Fusarium* wilt, and crown rot, caused by *Phytophthora capsici*, *Fusarium oxysporum,* and *Pythium ultimum*, respectively (Cho et al., 2002; Cho et al., 2007). Previously, endophytic bacteria have been reported

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to show antifungal activity against plant pathogenic fungi such as, *F. oxyporum* and *Rhizoctonia solani* in cotton; *Verticillium dahliae*, *Verticillium alboatrum*, and *Rhizoctonia solani* in potato; *Sclerotium rolfsii* in beans; *Verticillium longisporum*, *Rhizoctonia solani*, *F. oxyporum*, and *Phythium ultimum* in balloon flower; and *Rhizoctonia solani*, *Paenibacillus polymyxa*, *Bacilllus* sp., and *Pseudomonas poae* in ginseng (Chen et al., 1995; Cho et al., 2002; Graner et al., 2003; Berg et al., 2005; Seo et al., 2010). Endophytes may also be beneficial to the host plant by producing a variety of substances that participate in plant protection (Blanco et al., 2010; Chakraborty et al., 2010). Hence, the compounds produced by endophytic bacteria have a potential use in medicine, agriculture, and industry.

An investigation of the plant species inhabiting the southern mountainous regions of Korea reveals the total number of plant species mainly found in the mountain areas of Jirisan (JR) and Yarongsan (YR). The two representative mountains of this region are home to an estimated 4,000 plant species (Kim and Song, 2011). The plant diversity also influences the microbial diversity of forest soils (Kang et al., 2010).

Endophytes are microorganisms that thrive within the living tissues of plants. In most cases, the microbial relationship with the host plant is symbiotic or mutualistic. The microorganisms are capable of synthesizing bio-active compounds that can be used by the plant as a defense mechanism against pathogenic fungi and bacteria (Ryu et al., 2000). Endophytes also promote plant growth, suppress pathogens, detoxify contaminants, solubilize phosphate, and assist in nitrogen fixation in plants (Zheng et al., 2005). Recent studies of endophytic bacteria have focused on their roles within plants with relation to plant nutrition, pollutant catabolism, stress or defense responses, and invasion of pathogens (Cho et al., 2002).

Several endophytic bacterial species can be isolated from a single plant. Gram-positive and Gram-negative endophytic bacteria have been isolated from different tissues of various plants (Zinniel et al., 2002). Endophytic Gram-positive bacteria, such as *Bacillus* sp., have been isolated from cotton, cucumber root, and balloon flower root plant (Dujiff et al., 1997; Reva et al., 2002; Lima et al., 2005). *Paenibacillus polymyxa* has been isolated from the Korean ginseng root (Jeon et al., 2003). Endophytic *Bacillus halmapalus* is associated with the rice plant, where the bacterium is harmless to rice plant and promotes plant growth by penetration (Rosemblueth and Martimez-Romero, 2006). Continuously, genetic diversity among endophytic populations of crop plants has been monitored by PCR-based techniques, revealing a range of organisms that belong to several distinct phylogenetic groups (Garbeva et al., 2001; Cho et al., 2002; Graner et al., 2003; Cho et al., 2007; Asraful-Islam et al., 2010). The endophytic bacterial diversity of the balloon flower, *Platycodon gradiflorum*, was characterized based on 16S ribosomal DNA (rDNA) gene sequencing. At different ages of the plant, its roots possessed antimicrobial activities that could be directed towards the use of endophytic bacteria as bio-control agents against plant pathogens (Asraful-Islam et al., 2010).

In the present study, we demonstrated the biodiversity of endophytic bacteria isolated from deodeok plants root of different ages collected from Jirisan and Yarongsan areas in Korea. The isolated endophytic bacteria were evaluated for extracellular hydrolytic enzymes and antimicrobial activities.

MATERIALS AND METHODS

Isolation of endophytic bacteria from deodeok roots

Endophytic bacteria were isolated from two-, five-, and seven-year-old deodeok (*C. lanceolata*) roots. A total of ten replicated random root samples were collected from two mountainous areas (Jirisan and Yarongsan) located on the southern region of South Korea. Detail geographical information is available at <http://en.wikipedia.org/wiki/ List_of_mountains_of_Korea>. Yarongsan is called a same name as Waryongsan, Gyeongsangnam-do in the list. The roots samples were surface sterilized with 1% sodium hypochlorite for 10 min to remove epiphytic bacteria (Cho et al., 2002; Cho et al., 2007). The external root portion was chopped off (0.5 cm) from the margin with a sterile blade. Later, the root tissue was macerated in a sterile porcelain mortar along with sterile phosphate buffer (10 mM; pH 7.2). The root extracts were spread on tryptic soy agar (TSA) plates (Difco, NJ, USA) and incubated at 28 and 37°C for 48 h. The bacterial colonies that appeared on TSA were initially screened and grouped based on their morphological characteristics (Cho et al., 2002).

Test microorganisms and growth conditions *E. coli*

Escherichia coli (DH5α) was required for rDNA sequencing and cultured in Luria-Bertani (LB) broth (Difco, NJ, USA) at 37°C. The plant fungal pathogens, viz., *R. solani*, *Phythium ultimum*, *Phytophthora capsici*, and *F. oxysporum*, were provided by the Laboratory of Phytopathology, Gyeongnam Agricultural Research and Extension Services, Jinju, Korea. The fungi were cultured on potato dextrose agar (PDA, Difco, NJ, USA) medium at 28°C. Human pathogens, viz., *Escherichia coli* KCTC 1682, *Salmonella enteritidis* KCTC 12456, *S. typhimurium* KCTC 1925, *Shigella flexineri* KCTC 2008, *Shigella sonnei* KCTC 2518, *B. cereus* KCTC 3624, *Listeria innocula* KCTC 3586, *Listeria ivanovii* KCTC 3444, *Listeria monocytogenes* KCTC 3569 and *Staphylococcus aureus* KCTC 1621 were collected from the Korean Collection for Type Cultures (KCTC) in Biological Resource Center, Daejeon 305-333, Korea. TSA medium was used for the isolation of deodeok endophytic bacteria. These bacteria were also cultured on potato dextrose agar (PDA, Difco, NJ, USA) medium at 28 and 37°C to determine the optimal temperature for growth.

Analysis of 16S rDNA of the endophytic bacteria

The DNA from endophytic bacteria was extracted with the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, Suwon, Korea). The 16S rDNA gene was amplified by PCR using endophytic bacterial DNA as the template and universal primers (877F: 5′-CGGAGAGTTTGATCCTGG-3′, 878R: 5′-TACGGCTACCTTGTTAGCGAC-3′) (Cho et al., 2002; Cho et al., 2007). The amplification reaction mix also included Super-Therm DNA polymerase (JMR, Side Cup, Kent, UK), 1.5 mM $MgCl_2$, and 2

mM dNTP in a final reaction volume of 50 µl. PCR was conducted for 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s, with a final extension of 72°C for 10 min. PCR products that were obtained were cloned into the pGEM-T Easy vector (Promega, WI, USA) and sequenced using the PRISM Ready Reaction Dye terminator kit (Perkin-Elmer Corp., Norwalk, CT, USA) and an ABI model 3100 automated DNA sequencer (ABI, CA, USA). All reference sequences were obtained from the National Center for Biotechnology Information (NCBI) and Ribosomal Database Project (RDP) databases. The 16S rDNA similarity sequence searches were performed using the BLASTn and PSI-BLAST tools on the NCBI website (McGinnis and Madden, 2004). Sequences were aligned using the multiple sequence alignment program CLUSTAL W (Tompson et al., 1994). Gaps and positions with ambiguities were excluded to perform the phylogenetic analysis using neighbor-joining methods (Saito and Nei, 1987). Bootstrap analysis was performed using data re-sampled 1,000 times using the DNAMAN analysis system (Lynnon Biosoft, Quebec, Canada).

Assay of the hydrolytic enzyme activity of the endophytic bacteria

The agar diffusion method was used to assess the extracellular hydrolytic enzyme activities of the isolated deodeok root endophytic bacteria. The endophytic isolates were grown on different enzyme indicator media containing 0.5% (w/v) each of the following: carboxylmethylcellulose (CMC), oat spelt xylan (OSX), locust bean gum (LBG), and lichen to detect cellulase, xylanase, mannase and lichinase activity, respectively. The presence of each respective enzyme was indicated as a yellow halo surrounded by a red background on plates that were flooded with Congo red (0.5%) solution for 30 minutes (An et al., 2005). The pectinase and amylase enzymes in the bacterial isolates were detected with an indicator medium containing (1% w/v) polygalacturonic acid and starch as substrates. The amylase and pectinase activities were visualized as halo zones when the plates were flooded with 0.1% toluidine blue and potassium iodide, respectively (Park et al., 2000). The activity of other enzymes, such as protease, lipase, esterase, DNase, and chitinase, was also detected in the isolates using skim milk (2.5% v/v), tricaprylin (1% v/v), tributyrin (1% v/v), Difco DNAse medium and chitin (0.5% w/v), respectively, as substrates.

Antifungal activity of the endophytic bacteria against plant pathogens

An *in vitro* bioassay was conducted to evaluate the antagonistic activities of the bacterial isolates from the deodeok roots against *P. capsici*, *F. oxysporum*, *R. solani* and *P. ultimum*. Using the paper disk method, the disks were inoculated with 10 µl of bacterial suspension containing approximately 10^8 cfu ml^{-1} bacteria (Carruthers et al., 1994). The paper disks containing the bacteria were placed on the surface of the agar plates and incubated in an inverted position at 28°C for 48 h. The antifungal activity was scored by measuring the diameter of the clearance zone due to fungal growth inhibition. The detail methods were described in Cho et al. (2002) and Cho et al. (2007).

Antibacterial activity of endophytic bacteria against human pathogens

An *in vitro* bioassay was performed to evaluate the antibacterial activity of isolated endophytic bacteria against human pathogenic bacteria, including the following strains: *E. coli* KCTC 1682, *S. enteritidis* KCTC 12456, *S. typhimurium* KCTC 1925, *S. flexineri* KCTC 2008, *S. sonnei* KCTC 2518, *B. cereus* KCTC 3624, *L. innocula* KCTC 3586, *L. ivanovii* KCTC 3444, *L. monocytogenes* KCTC 3569 and *S. aureus* KCTC 1621. Using the paper disk met-hod, the disks were inoculated with 10 µl of bacterial suspension, containing approximately 10^8 cfu ml^{-1} of bacteria, and were placed on the surface of the agar plates. The plates were incubated in an inverted position at 28°C for 48 h, and the antibacterial activity was measured by the diameter of the clearance zone. The detail methods were described in Cho et al. (2002) and Cho et al. (2007).

Statistical analysis

Statistical analysis was performed for 16s rDNA homology, assay of enzyme activity, antifungal activity, and antibacterial activity by two-way analysis of variance (ANOVA) and Tukey's test (alpha =0.05) for randomized complete block design (RCBD) using SAS program (SAS9.1, SAS Institute Inc., Cary, NC).

RESULTS

16S rDNA homology analysis

PCR amplification of the 16S rDNA from the endophytic bacteria yielded a 1.5 kb product that was further cloned and sequenced. The 16S rDNA sequences were 98 to 99% similar when analyzed using the BLASTn and PSI-BLAST search tools. The homology analysis of the 16S rDNA sequences, performed with the bacterial isolates from the Jirisan region, were compared to the following strains in the NCBI database: *Enterobacter* sp. WAB1938 (AM184277), *Bacillus polyfermenticus* GR010 (DQ659145), *B. subtilis* TUL322 (JF412545), *S. saprophyticus* ATCC 15305 (AP008934), *Micrococcus* sp. TUT1210 (AB188213), *B. licheniformis* (AY971527), *Microbacteriaceae bacterium* KVD-1921 (DQ490452), *Bacillus sporothermodurans* (BSU49079), *Bacillus pumilus* BPT-18 (EF523475) and *Bacillus clausii* ATCC21537 (AB201796).

Homology analysis of the 16S rDNA sequences, isolated from the bacterial strains of Yarongsan area, matched well with the following strains in the NCBI database: *B. cereus* BGSC 6A5 (AY224388), *Enterobacter* sp. WAB1938 (AM184277), *Pseudomonadaceae bacterium* KVD-1959-04 (DQ490328), *Bacillaceae bacterium* KVD-unk-56 (DQ490420), *Micrococcus* sp. TUT1210 (AB188213), *M. bacterium* KVD-1921-08 (DQ490452), *B. polyfermenticus* GR010 (DQ659145), *B. licheniformis* (AY971527), *B. subtilis* TUL322 (JF412545) and *Pseudo-monas* sp. SPF-1 (DQ272493). Among the bacterial strains, three strains matched with *Enterobacter* sp. WAB1938 (AM184277), *Micrococcus* sp. TUT1210 (AB188213), and *M. bacterium* KVD-1921 (DQ490452) and were found in all three samples and from both the collection sites.

The diversity of endophytic bacteria from two growing areas, namely Jirisan and Yarongsan, was studied in two-, five, and seven-year-old deodeok root samples. A total of 10 different bacterial strains were isolated from approximately 120 bacterial colonies that were cultured from the interior of deodeok roots obtained from both growing areas (Table 1). The sequences of isolated clones have

been uploaded to GeneBank in NCBI. All 40 strains (20 of JR and 20 of YR) accession numbers (JQ229685-JQ229724) are described in Table 1.

Two-year-old deodeok root samples, collected from Jirisan, consisted of seven different bacterial strains obtained from 40 bacterial colonies (Table 1). The largest proportion of the bacterial colonies (10 colonies) was closely related to *Enterobacter* sp. WAB1938 (AM184277). Five-year-old deodeok root samples had seven different bacterial strains obtained from 40 bacterial colonies (Table 1). The largest proportion of the bacterial colonies (seven colonies) was closely related to *Micrococcus* sp. TUT1210 (AB188213). Seven-year-old deodeok root samples consisted of six different bacterial strains obtained from 40 colonies (Table 1), with the largest proportion of the bacterial colonies (10 colonies) closely related to *M. bacterium* KVD-1921 (DQ490452).

Among the Yarongsan samples, the two-year-old deodeok roots contained six different bacterial strains obtained from 40 bacterial colonies (Table 1). The largest proportion of the bacterial colonies (six colonies) was closely related to *Enterobacter* sp. WAB1938 (AM184277) and *B. sporothermodurans* (BSU49079). Five-year-old deodeok root samples yielded six different bacterial strains obtained from 40 bacterial colonies (Table 1), of which a large number of the bacterial colonies (10 colonies) was related to *M. bacterium* KVD-1921-08 (DQ490452). Seven-year-old deodeok root samples had the most diversity with eight different bacterial strains obtained from 40 colonies (Table 1), of which the largest proportion of the bacterial colonies (six colonies) was also closely related to *M. bacterium* KVD-1921 (DQ490452).

Phylogenetic placement of endophytic bacteria based on 16S rDNA sequences

Phylogenetic placement of the isolated deodeok endophytic bacterial strains was explored using the principle of 16S rDNA sequencing (Figure 1). Three types of clusters were found, including, High G+C Gram-positive bacteria, Actinobacteria (HGCGPB), Low G+C Gram-positive bacteria, *Firmicutes* (LGCGPB), and *Proteobacteria*. In the Jirisan samples, the population of LGCGPB was the most abundant. The population of LGCGPB increased in the two-year-old to seven-year-old Jirisan samples (Figure 2). *Proteobacteria,* HGCGPB, and LGCGPB were highest in the two-year-old, six-year-old and seven-year-old Yarongsan samples, respectively.

The HGCGPB cluster, found in the Jirisan samples, was related to *Micrococcus* sp. TUT1210 (JR2-5, JR5-3 and JR7-6) and *M. bacterium* KVD-1921 (JR2-7, JR5-6, and JR7-5), while the LGCGPB cluster was closely related to *B. subtilis* MA139 (JR2-3, JR5-5 and JR7-2), *B. polyfermenticus* GR010 (JR2-2), *B. licheniformis* (JR2-6 and JR7-4), *B. pumilus* BPT-18 (JR5-4 and JR7-2), *B. sporothermodurans* (JR5-2 and JR7-1), *B. clausii* ATCC21537 (JR5-7) and *S. saprophyticus* ATCC 15305 (JR5-2 and JR7-1). The *Proteobacteria* cluster was related to an uncultured bacterium (JR2-1, JR7-3 and JR5-1).

The HGCGPB cluster, found in the Yarongsan samples, was related to *Micrococcus* sp. TUT1210 (YR2-5, YR5-3 and YR7-6) and *M. bacterium* KVD-1921 (YR2-6, YR5-4, and YR7-5) while the LGCGPB cluster was closely related to *B. subtilis* MA139 (YR5-1 and YR7-4), *B. polyfermenticus* GR010 (YR5-1 and YR7-4), and *B. licheniformis* (YR5-2 and YR7-1). The *Proteobacteria* cluster was related to an uncultured bacterium (YR2-2, YR5-5 and YR7-7), *P. bacterium* KVD-1959-04 (YR2-3 and YR5-6) and *Pseudomonas* sp. SPF-1 (YR7-8).

The hydrolytic enzyme activities of the isolated deodeok endophytic bacteria

The isolated deodeok endophytic bacteria were evaluated for the activity of hydrolytic enzymes, such as cellulase, xylanase, mannase, pectinase, amylase, protease, lipase, esterase, DNase, and chitinase (Table 2). In the Jirisan samples, *B. polyfermenticus* (JR2-1), *B. subtilis* (JR2-3, JR5-5), *B. licheniformis* (JR2-6) and *B. pumilus* (JR5-4, JR7-2) were positive for all of the enzymes tested. In the Yarongsan samples, *B. polyfermenticus* (YR5-1, YR7-4), *B. licheniformis* (YR5-2, YR7-1) and *B. subtilis* (YR7-2) exhibited these enzyme activities.

Protease was the most active enzyme detected in the bacterial populations in all samples in all of the Jirisan samples, showing an increase in activity from the two-year-old samples to the seven-year-old samples. In addition, protease was the most active enzyme detected in the bacterial populations isolated from the Yarongsan samples, showing an increase in activity from the two-year-old samples to the seven-year-old samples; however, in the two-year-old samples of YR, DNase and chitinase were the most active enzymes in these populations (Figure 3).

Antibacterial activity of endophytic bacteria against human pathogens

The antibacterial activity of the isolated deodeok endophytic bacteria was evaluated against the tested several human pathogens (Table 3 and Figure 4). Among the bacterial isolates of the Jirisan samples, *B. subtilis* (JR2-3, JR5-5) showed antibacterial activity against the tested human pathogens. *B. saprophyticus* (JR2-4), *Micrococcus* sp. (JR2-5, JR5-3, JR7-6), *M. bacterium* (JR2-7, JR5-6, JR7-5), and *B. clausii* (JR5-7) were also exhibited antibacterial activity against the tested pathogens. Among the bacterial isolates of the Yarongsan samples, antibacterial activity was observed with *B. subtilis* (YR7-2). *Micrococcus* sp. (YR2-6, YR5-3, YR7-6) and *M. bacterium* (YR2-5, YR5-4, YR7-5) strains did not show any antibacterial activity.

Among the bacterial strains isolated from the two-year-old Jirisan samples, antibacterial activity was highest

Table 1. Similarity data of 16S rDNA sequences from the endophytic bacteria isolated from the interior of deodeok (*C. lanceolata*) roots.

Isolate (accession number)	Number of isolate	Phylum	Nearest relative* (Accession number)	Similarity (%)
2 years				
Jirisan				
JR2-1 (JQ229685)	10	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
JR2-2 (JQ229686)	2	LGCGPB†	*Bacillus polyfermenticus* GR010 (DQ659145)	99
JR2-3 (JQ229687)	5	LGCGPB	*Bacillus subtilis* TUL322 (JF412545)	99
JR2-4 (JQ229688)	2	LGCGPB	*Staphylococcus saprophyticus* ATCC 15305 (AP008934)	99
JR2-5 (JQ229689)	3	HGCGPB‡	*Micrococcus* sp. TUT1210 (AB188213)	99
JR2-6 (JQ229690)	2	LGCGPB	*Bacillus licheniformis* (AY971527)	99
JR2-7 (JQ229691)	6	HGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
Yarongsan				
YR2-1 (JQ229705)	5	LGCGPB	*Bacillus cereus* BGSC 6A5 (AY224388)	99
YR2-2 (JQ229706)	6	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
YR2-3 (JQ229707)	6	Proteobacteria	*Pseudomonadaceae bacterium* KVD-1959-04 (DQ490328)	98
YR2-4 (JQ229708)	3	LGCGPB	*Bacillaceae bacterium* KVD-unk-56 (DQ490420)	99
YR2-5 (JQ229709)	7	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99
YR2-6 (JQ229710)	3	LGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
5 years				
Jirisan				
JR5-1 (JQ229692)	3	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
JR5-2 (JQ229693)	6	LGCGPB	*Bacillus sporothermodurans* (BSU49079)	98
JR5-3 (JQ229694)	7	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99
JR5-4 (JQ229695)	2	LGCGPB	*Bacillus pumilus* BPT-18 (EF523475)	99
JR5-5 (JQ229696)	4	LGCGPB	*Bacillus subtilis* TUL322 (JF412545)	98
JR5-6 (JQ229697)	6	HGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
JR5-7 (JQ229698)	2	LGCGPB	*Bacillus clausii* ATCC21537 (AB201796)	99
Yarongsan				
YR5-1 (JQ229711)	4	LGCGPB	*Bacillus polyfermenticus* GR010 (DQ659145)	99
YR5-2 (JQ229712)	5	LGCGPB	*Bacillus licheniformis* (AY971527)	99
YR5-3 (JQ229713)	7	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99
YR5-4 (JQ229714)	7	HGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
YR5-5 (JQ229715)	4	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
YR5-6 (JQ229716)	3	Proteobacteria	*Pseudomonadaceae bacterium* KVD-1959-04 (DQ490328)	98
7 years				
Jirisan				
JR7-1 (JQ229699)	6	LGCGPB	*Bacillus sporothermodurans* (BSU49079)	98

Table 1. Contd.

JR7-2 (JQ229700)	9	LGCGPB	*Bacillus pumilus* BPT-18 (EF523475)	99
JR7-3 (JQ229701)	1	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
JR7-4 (JQ229702)	1	LGCGPB	*Bacillus licheniformis* (AY971527)	99
JR7-5 (JQ229703)	10	HGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
JR7-6 (JQ229704)	3	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99
Yarongsan				
YR7-1 (JQ229717)	3	LGCGPB	*Bacillus licheniformis* (AY971527)	99
YR7-2 (JQ229718)	3	LGCGPB	*Bacillus subtilis* MA139 (DQ415893)	99
YR7-3 (JQ229719)	5	LGCGPB	*Bacillus cereus* BGSC A65 (AY224388)	99
YR7-4 (JQ229720)	5	LGCGPB	*Bacillus polyfermenticus* GR010 (DQ659145)	99
YR7-5 (JQ229721)	6	HGCGPB	*Microbacteriaceae bacterium* KVD-1921-08 (DQ490452)	98
YR7-6 (JQ229722)	3	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99
YR7-7 (JQ229723)	2	Proteobacteria	*Enterobacter* sp. WAB1938 (AM184277)	99
YR7-8 (JQ229724)	3	Proteobacteria	*Pseudomonas* sp. SPF-1 (DQ272493)	99

Table 2. The extracellular enzymes of the endophytic bacteria isolated from the interior of deodeok (*C. lanceolata*) roots

Isolate	Nearest relative* (accession number)	Extracellular hydrolysis enzyme activities†									
		Celllulase	Xylanase	Mannanase	Pectinase	Amylase	Protease	Lipase	Esterase	DNase	Chitinase
2 years											
Jirisan											
JR2-1	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
JR2-2	*B. polyfermenticus* (DQ659145)	+++	+++	+	++	+++	+++	+	+	++	+
JR2-3	*B. subtilis subtilis* TUL322 (JF412545)	++	+++	++	+	++	+++	++	++	+	+
JR2-4	*S. saprophyticus* ATCC 15305 (AP008934)	-	-	+++	-	-	-	+	++	-	-
JR2-5	*Micrococcus* sp. TUT1210 (AB188213)	-	-	--	-	--	++	+	-	-	-
JR2-6	*B. licheniformis* (AY971527)	++	+++	++	+	++	+++	+	++	++	+
JR2-7	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	+	-	-	-	-
Yarongsan											
YR2-1	*B. cereus* BGSC 6A5 (AY224388)	+	++	++	-	+++	+	-	+	+	+
YR2-2	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
YR2-3	*P. bacterium* KVD-1959-04 (DQ490328)	-	+	-	-	-	-	++	++	+	+
YR2-4	*B. bacterium* KVD-unk-56 (DQ490420)	-	-	-	-	-	-	-	-	+	-
YR2-5	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	++	+	-	-	-
YR2-6	*M. bacterium* KVD-1921-08 (DQ490452	-	-	-	-	-	-	-	-	+	-

Table 2. Contd.

5 years											
Jirisan											
JR5-1	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
JR5-2	*B. sporothermodurans* (BSU49079)	-	-	-	-	-	-	-	-	+	-
JR5-3	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	++	+	-	-	-
JR5-4	*B. pumilus* BPT-18 (EF523475)	++	++	+	+	++	+++	+	++	+	+
JR5-5	*B. subtilis subtilis* TUL322 (JF412545)	++	+++	++	+	++	+++	++	++	+	+
JR5-6	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	+	-	-	-	-
JR5-7	*B. clausii* ATCC21537 (AB201796)	-	-	-	-	-	+++	-	-	++	+
Yarongsan											
YR5-1	*B. polyfermenticus* GR010 (DQ659145)	+++	+++	+	++	+++	+++	+	+	++	+
YR5-2	*B. licheniformis* (AY971527)	++	++	++	+	+	+++	+	++	++	+
YR5-3	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	++	+	-	-	-
YR5-4	*M. bacterium* KVD-1921-08 (DQ490452	-	-	-	-	-	+	-	-	-	-
YR5-5	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
YR5-6	*P. bacterium* KVD-1959-04 (DQ490328)	-	-	-	-	-	-	++	++	+	+
7 years											
Jirisan											
JR7-1	*Bacillus sporothermodurans* (BSU49079)	-	-	-	-	-	-	-	-	+	-
JR7-2	*B. pumilus* BPT-18 (EF523475)	++	++	+	+	++	+++	+	++	+	+
JR7-3	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
JR7-4	*B. licheniformis* (AY971527)	++	++	++	+	+	+++	+	++	++	++
JR7-5	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	+	-	-	-	-
JR7-6	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	++	+	-	-	-
Yarongsan											
YR7-1	*B. licheniformis* (AY971527)	++	++	++	+	+	+++	+	++	++	+
YR7-2	*B. subtilis* MA139 (DQ415893)	++	++	++	+	++	+++	+	+	+	+
YR7-3	*B. cereus* BGSC 6A5 (AY224388)	+	++	+	-	+++	+	-	+	+	+
YR7-4	*B. polyfermenticus* GR010 (DQ659145)	+++	+++	+	++	+++	+++	+	++	++	+
YR7-5	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	+	-	-	-	-
YR7-6	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	++	+	-	-	-
YR7-7	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	-	-	-	-	+++
YR7-8	*Pseudomonas* sp. SPF-1 (DQ272493)	-	+	-	-	-	-	-	-	+	-

*Closest relative species in the 16S rDNA sequence database. When more than one sequence had the same similarity, only the accession number of the first sequence is given. †Size of halos formed around bacterial colonies on agar media. -, Implies no halo zone which indicates no enzyme activity; +, implies 2 mm diameter of the halo zone which indicates no enzyme activity; ++, implies no halo zone which indicates no enzyme activity; +, implies 4 mm diameter of the halo zone which indicates no enzyme activity; +++, implies no halo zone which indicates no enzyme activity; +, implies 6 mm diameter of the halo zone which indicates no enzyme activity.

Table 3. *In vitro* anti-microbial activities against the human and plant pathogens by endophytic bacteria isolated from the interior of deodeok (*C. lanceolata*) roots.

Isolate	Nearest relative*	*In vitro* inhibitory activities†													
		Human pathogenic bacteria‡										Plant pathogenic fungi§			
		Eco	***Sen***	***Sty***	***Sfl***	***Sso***	***Bce***	***Lin***	***Liv***	***Lmo***	***Sau***	***Pca***	***Fox***	***Rso***	***Pul***
2 years															
Jirisan															
JR2-1	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.4	10.9	-	-	-	8.4	-	-	-
JR2-2	*B. polyfermenticus* (DQ659145)	-	11.2	-	-	-	9.3	8.5	13.6	-	10.0	8.7	11.3	8.2	-
JR2-3	*B. subtilis subtilis* TUL322 (JF412545)	8.3	8.7	12.9	11.6	9.0	14.2	14.5	10.4	12.8	11.3	12.4	17.8	14.2	-
JR2-4	*S. saprophyticus* ATCC 15305 (AP008934)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JR2-5	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JR2-6	*B. licheniformis* (AY971527)	-	-	8.8	-	-	-	13.5	9.4	9.6	10.6	8.2	9.0	10.2	-
JR2-7	*M. bacterium KVD-1921-08* (DQ490452)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yarongsan															
YR2-1	*B. cereus* BGSC 6A5 (AY224388)	-	-	-	-	-	-	-	-	-	10.4	-	9.8	8.2	10.8
YR2-2	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.8	10.7	-	-	-	8.6	-	-	-
YR2-3	*P. bacterium* KVD-1959-04 (DQ490328)	-	-	-	-	-	-	11.2	-	-	-	-	11.2	-	-
YR2-4	*B. bacterium* KVD-unk-56 (DQ490420)	-	9.4	10.3	-	-	10.2	11.6	-	11.0	11.2	-	10.0	9.8	8.4
YR2-5	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YR2-6	*M. bacterium* KVD-1921-08 (DQ490452	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5 years															
Jirisan															
JR5-1	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.2	10.2	-	-	-	8.2	-	-	-
JR5-2	*B. sporothermodurans* (BSU49079)	-	-	-	-	-	8.4	-	-	-	-	-	-	-	-
JR5-3	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JR5-4	*B. pumilus* BPT-18 (EF523475)	-	-	-	-	-	11.0	-	12.0	8.8	-	9.3	10.8	11.2	9.3
JR5-5	*B. subtilis subtilis* TUL322 (JF412545)	8.5	8.8	13.2	11.4	9.2	14.8	14.6	11.2	12.6	12.3	12.4	16.6	14.6	-
JR5-6	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JR5-7	*B. clausii* ATCC21537 (AB201796)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yarongsan															
YR5-1	*B. polyfermenticus* GR010 (DQ659145)	-	11.2	-	8.2	10.0	10.6	9.6	12.4	-	12.2	9.0	11.6	9.2	-
YR5-2	*B. licheniformis* (AY971527)	-	-	9.0	-	-	-	12.8	10.0	11.2	10.4	8.4	9.2	9.8	-
YR5-3	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YR5-4	*M. bacterium* KVD-1921-08 (DQ490452	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YR5-5	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.2	9.8	-	-	-	8.6	-	-	-
YR5-6	*P. bacterium* KVD-1959-04 (DQ490328)	-	-	-	-	-	-	11.2	-	-	-	-	11.4	-	-

Table 3. Contd.

7 years															
Jirisan															
JR7-1	*Bacillus sporothermodurans* (BSU49079)	-	-	-	-	-	8.3	-	-	-	-	-	-	-	-
JR7-2	*B. pumilus* BPT-18 (EF523475)	-	-	-	-	-	10.8	-	12.2	9.6	-	9.6	10.4	11.6	10.3
JR7-3	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.2	10.4	-	-	-	8.5	-	-	-
JR7-4	*B. licheniformis* (AY971527)	-	-	8.8	-	-	-	12.6	10.4	11.8	9.6	8.5	9.0	10.4	-
JR7-5	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JR7-6	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Yarongsan															
YR7-1	*B. licheniformis* (AY971527)	-	-	10.0	-	-	9.8	11.6	10.9	12.2	10.2	11.8	9.2	10.2	-
YR7-2	*B. subtilis* MA139 (DQ415893)	8.4	10.8	11.2	11.2	8.8	14.6	14.0	10.4	10.2	11.0	12.4	17.0	14.2	-
YR7-3	*B. cereus* BGSC 6A5 (AY224388)	-	-	-	-	-	-	-	-	-	11.4	-	9.6	8.4	-
YR7-4	*B. polyfermenticus* GR010 (DQ659145)	-	11.4	-	9.8	-	10.8	10.6	13.4	-	13.2	8.8	9.0	10.2	-
YR7-5	*M. bacterium* KVD-1921-08 (DQ490452)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YR7-6	*Micrococcus* sp. TUT1210 (AB188213)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
YR7-7	*Enterobacter* sp. WAB1938 (AM184277)	-	-	-	-	-	8.6	11.2	-	-	-	8.3	-	-	-
YR7-8	*Pseudomonas* sp. SPF-1 (DQ272493)	-	10.0	-	-	-	-	-	-	-	-	-	12.4	-	13.2

*Closest relative species in the 16S rDNA sequence database. When more than one sequence had the same similarity, only the accession number of the firstsequence is given. †The antimicrobial activities were estimated by measuring the diameter of the clear zone (including paper disks, 8 mm diameter) of growth inhibition. ‡Human pathogenic bacteria: *Eco, Escherichia coli* KCTC 1682; *Sen, Salmonella enterica* KCTC 12456; *Sty, Salmonella typhimurium* KCTC 1925; *Sfl, Shigella flexineri* KCTC 2008; *Sso, Shigella sonnei* KCTC 2518; *Bce, Bacillus cereus KCTC 3624; Lin, Listeria innocula* KCTC 3586; *Liv, Listeria ivanovii* KCTC 3444; *Lmo, Listeria monocytogenes* KCTC 3569; *Sau, Staphylococcus aureus* KCTC 1621. §Plant pathogenic fungi: *Pca, Phytopthora capsii; Fox, Fusarium oxysporum; Rso, Rhizoctonia solani; Pul, Pythium ultimun.*

against *Listeria innocula* KCTC 3586, and the five-year-old and seven-year-old samples had the highest antibacterial activity against *B. cereus* KCTC 3624. Additionally, potent antibacterial activity was observed against *L. innocula* KCTC 3586 from the bacteria isolated from the two-year-old and five-year-old Yarongsan samples. In the seven-year-old Yarongsan samples, the population of bacteria showed the highest antibacterial activity against the human pathogen *Staphylococcus aureus* KCTC 1621.

Antifungal activity of endophytic bacteria against plant pathogens

An *in vitro* bioassay was conducted to evaluate the antagonistic activities of the isolated deodeok endophytic bacteria. Among the bacterial strains isolated from Jirisan samples, *B. pumilus* (JR5-4, 7-2) showed antifungal activity against all of the tested phytopathogenic fungi. *B. subtilis* (JR5-5) and *B. licheniformis* (JR2-6, JR7-4) showed antifungal activity against all of the tested phytopathogenic fungi, except *Pythium ultimum*. Among the bacterial strains isolated from Yarongsan samples, *B. cereus* (YR2-1) and *B. bacterium* (YR2-4) showed antifungal activity against all of the tested phytopathogenic fungi, except *P. capsici*. *B. polyfermenticus* (YR5-1, YR7-4), *B. licheniformis* (YR5-2, YR7-1) and *B. subtilis* (YR7-2) which showed antifungal activity against all of the tested phytopathogenic fungi, except *Pythium ultimum*.

Bacteria from the Jirisan samples had the highest antifungal activity against *P. capsici*, but *P. capsici* was absent in the two-year-old Jirisan samples. Moreover, *F. oxysporum*, *P. capsici* and *R. solani* were highest in the two-, five, and seven - year-old Yarongsan samples (Figure 5). *R. solani* was the most sensitive strain for seven-year-old samples from the Yarongsan site. Similarly, two-year-old samples from the Jirisan site showed highest activity against *P. capsii.* Other samples showed varying degrees of antifungal activity (Figure 5).

DISCUSSION

Our study demonstrates the diversity and antimic-

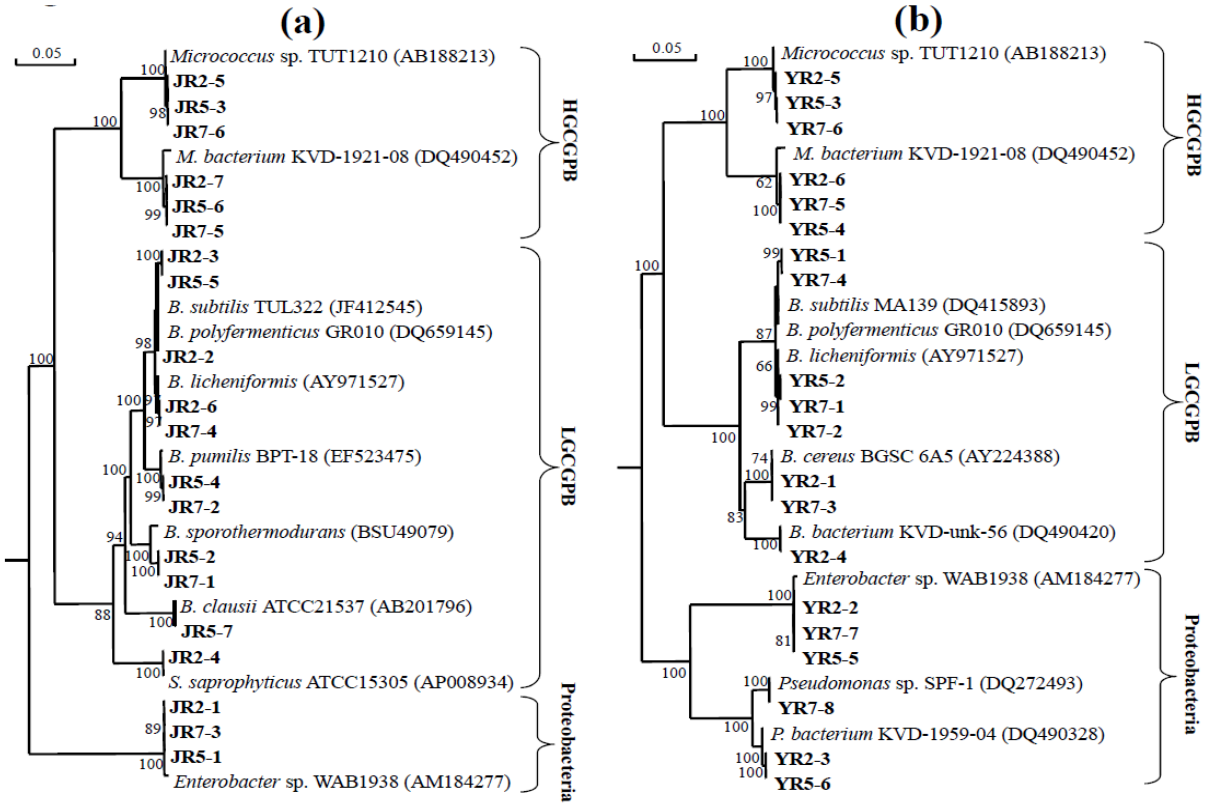

Figure 1. Phylogenetic analysis of 16S rDNA sequence derived from the endophytic bacteria of the deodeok roots from Jirisan (a) and Yarongsan (b). Numbers above each node are confidence levels (%) generated from 1,000 bootsrap trees. The scale bar is in fixed nucleotide substations per sequence position.

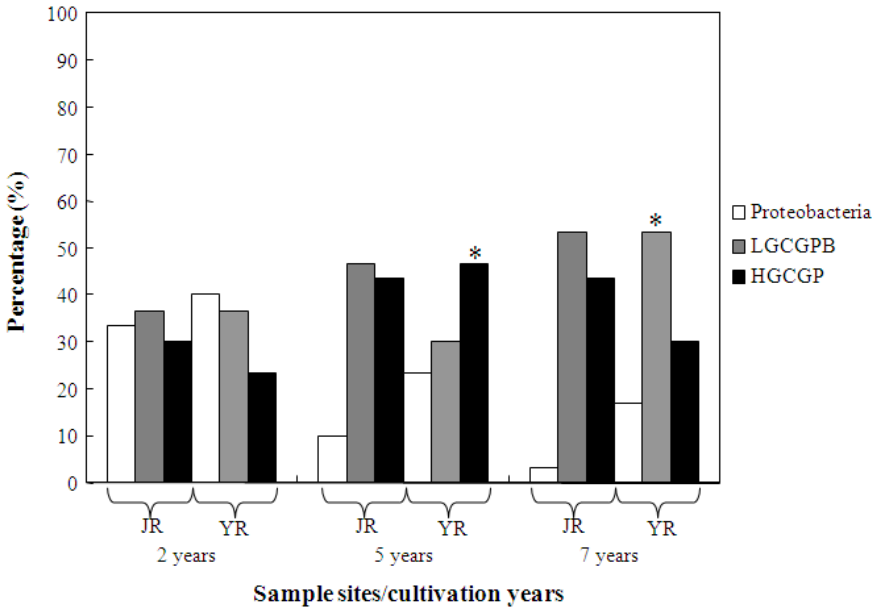

Figure 2. Distribution of the 16S rDNA sequences of isolates in each of the two sample sites: Jirisan (JR) and Yarongsan (YR) and three cultivation years, two years, five years, and seven years. Numbers in square brackets provide the total number of the corresponding isolates for sample sites. Percentage of microcosm in each of the two sample sites and cultivation years were shown. *indicates that the percentage of microcosm was significantly different α=0.05) from other sample site at the given time.

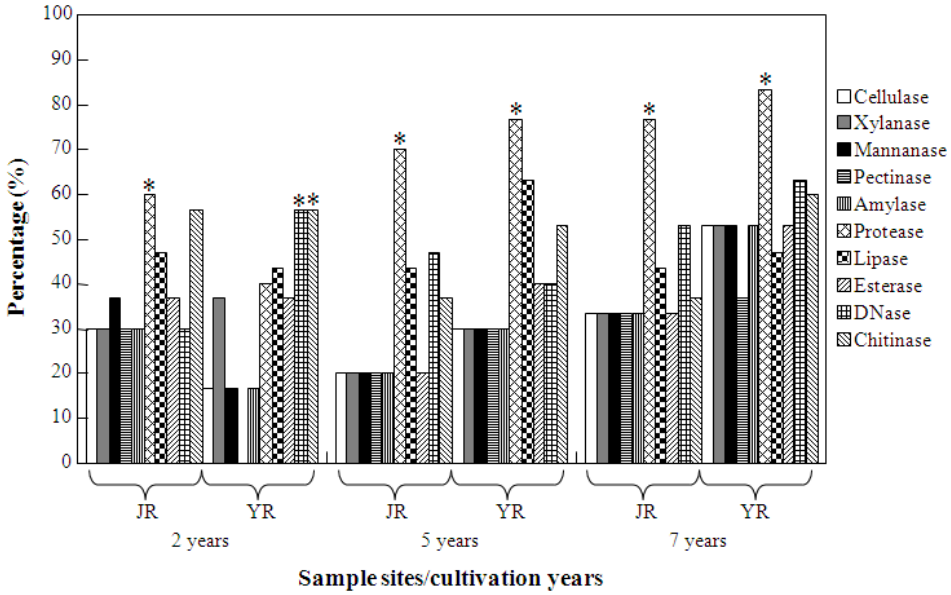

Figure 3. Distribution of the extracellular hydrolytic enzymes in endophytic isolates from deodeok roots of Jirisan (JR) and Yarongsan (YR) sites and their relation to root age. Numbers in square brackets provide the total number of the corresponding isolates for sample sites. Percentage of microcosm producing the extracellular hydrolysis enzymes in each of the two sample sites and cultivation years were shown. '*' indicates that the percentage of microcosm was significantly different *alpha*=0.05) from other sample site at the given time.

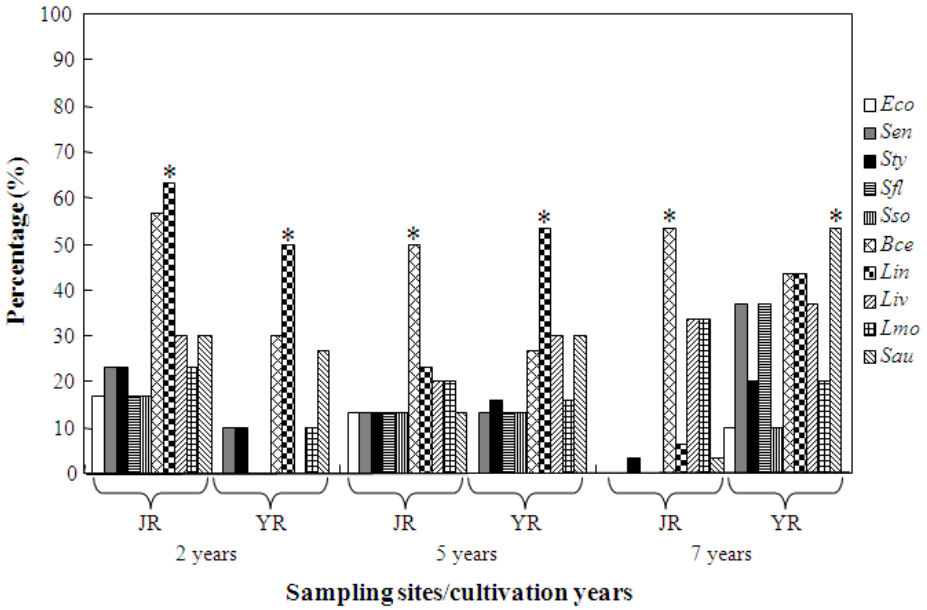

Figure 4. Distribution of the antibacterial activities against human pathogens in bacterial isolates obtained from sampling sites in Jirisan (JR) and Yarongsan (YR) and their relation to deodeok root age. Numbers in square brackets represent the total number of the isolates in the corresponding sample sites. Percentage of microcosm in each of the two sample sites and cultivation years are shown. Human pathogenic bacteria: Eco, *E. coli* KCTC 1682; Sen, *S. enterica* KCTC12456; Sty, *S. typhimurium* KCTC1925; Sfl, *S. flexineri* KCTC2008; Sso, *S. sonnei* KCTC2518; Bce, *B. cereus* KCTC3624; Lin, *L. innocula* KCTC3586; Liv, *L. ivanovii* KCTC3444; Lmo, *L. monocytogenes* KCTC3569; Sau, *S. aureus* KCTC1621. *indicates that the percentage of microcosm was significantly different (α=0.05) from other sample site at the given time.

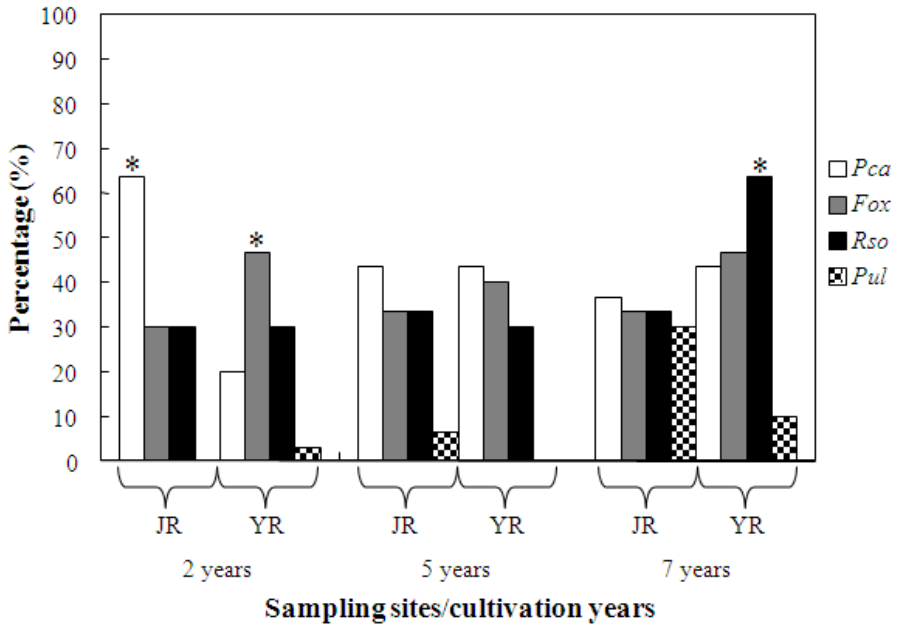

Figure 5. Distribution of the phyto-pathogenic antifungal activities in bacterial isolates obtained from Jirisan (JR) and Yarongsan (YR) sampling sites and related to deodeok root age. Numbers in square brackets indicate the total number of the isolates in the corresponding sample site. Percentage of microscosm in each of the two sample sites and cultivation years are shown. Plant pathogenic fungi: Pca, *P. capsici*; Fox, *F. oxysporum*; Rso, *R. solani*; Pul, *P. ultimun*.*' Indicates that the percentage of microcosm was significantly different (α=0.05) from other sample site at the given time.

robial activity of bacteria in deodeok roots of different ages from two sampling areas. Sequencing of the 16S rRNA genes from the endophytic bacteria, isolated from the deodeok roots, revealed the presence of High G+C Gram-positive bacteria (HGCGPB), low G+C Gram-positive bacteria (LGCGPB), and proteobacteria. The population of the LGCGPB was highest in the samples collected from Jirisan; concurrently, the LGCGBP population counts varied depending upon the age of the roots. The samples from Yarongsan showed the presence of Proteo-bacteria, HGCGPB, and LGCGPB where their numbers directly depended upon the root age. The HGCGPB populations were previously isolated and identified from wheat, potato crops, and radish (Combs and Franco, 2003; Coon and Franco, 2004; Garbeva et al., 2001; Seo et al., 2010).

The results regarding the diversity of endophytic bacteria, isolated from the interior of the deodeok roots, indicate that the bacterial population and types depend upon the cultivation period and the sampling site. Among the bacterial strains isolated in this study, *Bacillus* spp. was dominant in all three samples from two different locations. Several studies on endophytic bacteria in the roots of ginseng, cotton, sweet corn, canola, and balloon flower were previously performed. For instance, 13 isolates belonging to the LGCGPB and Proteobacteria groups were assessed for their antifungal activity against *R. solani*, *Paenibacillus polymyxa*, *Bacilllus* sp., and *P. poae* in ginseng (Cho et al., 2007). The root rot (*P. polymyxa*) was detected in Korean ginseng and involved in growth promoting rhizobacterium (Jeon et al., 2003). The endophytic *Bacillus* spp. has also been isolated from cotton, cucumber, balloon flower, and citrus root (Dujiff et al., 1997; Reva et al., 2002; Lima et al., 2005). The endophytic *Bacillus* sp. was also isolated from rice plants, which had promoted plant growth by penetration (Zheng et al., 2006) and similar phylogenic groups of endophytic bacterial populations in potato (Garbeva et al., 2001).

The hydrolytic enzyme activity among the endophytic bacteria obtained from the deodeok root samples showed varying enzyme activity with root age and sampling site. The hydrolytic enzymes aid the entry of endophytic bacteria into the plant's roots. Plant cell wall hydrolytic enzymes also play an important role in plant-microbe interactions and intercellular colonization of microorganisms in the plant root. Bacteria could enter the root interior by hydrolyzing cell wall-bound cellulose and also enter through auxin-induced tumors, water flow, and wounds or at sites of lateral root branching (Al-Mallah et al., 1987). Similarly, the presences of different levels of cellulase and pectinase activity in different bacterial isolates showed a potential for intercellular and intracellular colonization (Verma et al., 2001). In our study, protease activity was highest in bacterial populations isolated from all the Jirisan samples and increased with root age, as observed in the two-year-old to seven-year-old samples. In the Yarongsan bacterial samples, protease

activity was highest in the two-year-old and seven-year-old samples, but in the five-year-old samples, DNase and chitinase activity were highest. These potential endophytic bacteria can be harnessed for the production of hydrolytic enzymes for biotechnology applications.

Between the bacterial isolates of the Jirisan and Yarongsan samples, *B. subtilis* showed antibacterial activity against human pathogens. *Bacillus* sp. (BF1-2 and BF3-5) can be used for the control of *Salmonella* spp. and other human pathogens. Among the isolated endophytic bacteria from the Jirisan deodeok root samples, *B. pumilus* (JR5-4, 7-2) showed antifungal activity against the tested phytopathogenic fungi, except for *B. polyfermenticus* (JR2-2, JR2-3), while *B. subtilis* (JR5-5) and *B. licheniformis* (JR2-6, JR7-4) showed antifungal activity against the tested phytopathogenic fungi, except for *Pythium ultimum*. Among the bacterial isolates from the Yarongsan root samples, *B. cereus* (YR2-1) and *B. bacterium* (YR2-4) showed antifungal activity against the tested phytopathogenic fungi, except for *Phytophthora capsici*. Besides, *B. polyfermenticus* (YR5-1, YR7-4), *B. licheniformis* (YR5-2, YR7-1) and *B. subtilis* (YR7-2) showed antifungal activity against all of the tested phytopathogenic fungi, except for *Pythium ultimum*. *Bacillus* sp. CY22 was isolated from the balloon flower root; this strain showed antifungal activity against several plant pathogens by producing the antibiotic iturin A, which is similar to the phytochemicals found in the deodeok plant (Cho et al., 2002). In the Jirisan samples, the bacterial populations exhibited the highest antifungal activity against *P. capsici*, but bacteria showing antifungal activity against *P. capsici* were absent in the two-year-old Jirisan samples. Bacterial populations showing antifungal activity against *F. oxysporum*, *P. capsici*, and *R. solani* was high in all of the root samples that were collected from Yarongsan at all ages. The endophytic bacteria have a potential to inhibit the following plant pathogenic fungi: *F. oxyporum* and *R. solani* in cotton; *Verticillium dahliae*, *Verticillium alboatrum* and *Rhizoctonia solani* in potato; *Sclerotium rolfsii* in beans; and *Verticillium longisporum* in *Brassica napus* (Berg et al., 2005; Chen et al., 1995; Graner et al., 2003). The antifungal endophytes are beneficial to the host plant because they help protect the plant against fungal infection. However, endophytic bacteria mediate resistance to disease, and signals exist to mediate cross-talk between the endophytic bacteria and their hosts.

For future studies, we will prepare to analyse the quantitative comparisons from un-cultured isolates of each JR and YR samples with metagenomic tools because it will be a good method to explore and to justify a more proper comparison on abundances. From cultured microorganisms in a particular medium, it used to generate the statistical parameters which were too weak and the limited amount of selected isolates was obtained. As analysis of 16s rRNA gene has the current limits, future research requires particular cultivation independent methods such as clone libraries or pyro-sequencing, in order to retrieve a more realistic view of the larger fraction of microbial diversity in the particular environment.

In conclusion, we demonstrated that there are differences in the diversity of endophytic bacteria from deodeok roots and that these differences depend on the year and area of cultivation. The population of LGCGPB was highest in all three of the cultivation years of the Jirisan root samples and the seven-year-old root samples from Yarongsan. Proteobacteria and HGCGPB populations were highest in the two-year-old and five-year-old roots from Yarongsan, respectively. Among the isolated bacterial strains from Jirisan, *B. polyfermenticus* (JR2-1), *B. subtilis* (JR2-3, JR5-5), *B. licheniformis* (JR2-6), and *B. pumilus* (JR5-4, JR7-2) were positive for the hydrolytic enzymes tested. *B. polyfermenticus* (YR5-1, YR7-4), *B. licheniformis* (YR5-2, YR7-1), and *B. subtilis* (YR7-2) showed enzyme activity among the Yarongsan root samples. Among the bacterial isolates from the Jirisan and Yarongsan root samples, *B. subtilis* showed antibacterial activity against human pathogens. Some of the bacteria isolated from the Jirisan deodeok roots showed antifungal activity. For instance, *B. pumilus* (JR5-4, JR7-2) showed antifungal activity against all of the tested phytopathogenic fungi, except for *B. polyfermenticus* (JR2-2, JR2-3). *B. subtilis* (JR5-5) and *B. licheniformis* (JR2-6, JR7-4) showed antifungal activity against all of the tested phytopathogenic fungi, except for *Pythium ultimum*. Among the bacterial isolates from the Yarongsan roots, *B. cereus* (YR2-1) and *B. bacterium* (YR2-4) showed antifungal activity against all of the tested phytopathogenic fungi, except of the *Phytophthora capsici*. Besides, *B. polyfermenticus* (YR5-1, YR7-4), *B. licheniformis* (YR5-2, YR7-1) and *B. subtilis* (YR7-2) showed antifungal activity against all of the tested phytopathogenic fungi, except for *P. ultimum*. Therefore, the deodeok endophytic bacteria, including *B. subtilis*, *B. licheniformis*, *B. polyfermenticus*, and *B. pumilus*, from both the Jirisan and Yarongsan regions, can be used as potential biological antimicrobial agents for biological applications.

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