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Article

Endophytic Bacterial Diversity in the Young Radish and Their Antimicrobial Activity against Pathogens

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Endophytic bacteria have several ecological roles and can be used as biocontrol agents and also participate in antibiosis interactions. The diversity of endophytic bacteria associated with young radish (YR, *yeulmu*, *Raphanus sativus* L.) leaves and roots from Gyeongnam Agricultural Research and Extension Services in Jinju, Korea was investigated. A total of 264 colonies were isolated from the interior of YR leaves and roots. Phylogenetic analysis based on 16S rDNA sequences indicated that the isolates belonged to four major phylogenetic groups: high G+C Gram positive bacteria, low G+C Gram positive bacteria, *Proteobacteria*, and *Bacteroidetes*. Endophytic bacteria from the phylum *Proteobacteria* were predominant in the leaf (61.3%) and root (52.1%) samples. Most colonies that exhibited extracellular enzymatic activity belonged to the genus *Bacillus*, and *Bacillus subtilis* (YRL02, YRL07, YRR03, and YRR10) exhibited the stronger activities in extracellular enzyme such as amylase, cellulase, xylanase, mannase, PGAase, DNase, protease, and esterase than other colonies. In addition, *Enterobacter* sp. YRL01 and *B. subtilis* YRL02 had the highest amount of inhibitory action against human pathogenic bacteria, while *B. subtilis* YRR10 hand an inhibitory action against plant pathogenic fungi. Thus, these bacteria can be used as biocontrol agents against human and plant pathogens.

Key words: antimicrobial activity, endophytic bacteria, human pathogenic bacteria, microbial diversity, plant pathogenic fungi, young radish

Endophytes spend much, if not all, of their lives in association with host plants as latent or active organisms. The term "endophyte" usually denotes mycorrhizal fungi, which reside only partly within plant tissues [Mocali *et al.,* 2003], and fungi or bacteria that colonize the tissues of living plants but cause disease symptoms [Hallmann *et al*., 1997]. Many factors such as plant rotation, soil condition, and the presence of phytopathogens can influence the population structures of endophytic bacteria [Hallmann *et al.,* 1997; Granér *et al.,* 2003]. Endophytes promote plant growth and yield by suppression the pathogens and they may help to remove contaminants, solubilize phosphate, and contribute assimilable nitrogen to plants [Rosenblueth and Martinez-Romero, 2006]. Several studies have investigated endophytic bacteria as biological control agents due to their production of antimicrobial metabolites [Strobel *et al.,* 2004].

To date, estimations of endophytic bacterial species diversity have been largely based on culture techniques. Mundt and Hinkle [1976] reported as many as 46 different bacterial species from 27 plant species. There have also been many reports on the isolation and diversity of endophytic bacteria from various plant species, such as cotton, sweet corn cultivars [McInroy and Kloepper, 1995], pea cultivars [Elvira-Recuenco and van Vuurde, 2000], *Brassica napus* cultivars [Germida *et al.,* 1998; Siciliano and Germida, 1999; Granér *et al.,* 2003], and citrus cultivars [Araújo *et al.,* 2002]. In addition, genetic diversity among endophytic populations of crop plants

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has been monitored by PCR-based techniques, revealing a range of organisms that belong to several distinct phylogenetic groups [Garbeva *et al.*, 2001; Granér *et al.,* 2003]. Analyses of endophytic bacteria that colonize potato plants revealed five major groups: the *Proteobacteria*, the high G+C Gram positive bacteria (*HGCGPB*), the low G+C Gram positive bacteria (*LGCGPB*), the *Flexibacter Cytophaga Bacteroides* group, and the *Planctomycetales* groups [Reiter *et al.,* 2002]. In previous, Cho *et al.* [2007] reported 13 bacterial genera among 63 isolates from the interior roots of three ginseng plants by 16S rDNA gene analysis.

A general call exists for new antibiotics, chemotherapeutic agents, and agrochemicals that are highly effective, possess low toxicity, and have a minor environmental impact [Strobel and Daisy, 2003]. Often, endophytes are a source of these antibiotics. Natural products from endophytic microbes have been observed to inhibit or kill a wide variety of harmful disease-causing agents including, but not limited to, phytopathogenes and bacteria, fungi, viruses, and protozoans that affect humans and animals [Strobel and Daisy, 2003].

Radishes (*Raphanus sativus* L.) are an important vegetable crop in East Asia. Young radish (YR*, yeulmu*, *Raphanus sativus* L.) in particular is a major raw material of the Korean traditional fermented dish *kimchi.* The initial stage of *kimchi* fermentation is associated with several bacteria species: *Aeromonas* sp., *Erwina* sp., *Plesiomonas* sp., *Pseudomonas* sp., *Xenorhabdus* sp. and *Bacillus* sp. [An *et al.,* 1999].

In this study, the population structures of endophytic bacteria of the YR from Gyeongnam Agricultural Research and Extension Services in the Jinju area of Korea was investigated. The use of some endophytic species of the young radish as biological control agents against plant pathogenic fungi and human pathogenic bacteria was also investigated in this study, as well as the extracellular enzymatic activity of isolated endophytic bacteria of the young radish.

Materials and Methods

Microorganisms, plasmids and media. Endophytic bacteria were isolated from YR and cultured at 28 or 37°C in tryptic soy (TS) medium and number 3 medium (10 g polypeptone, 10 g glucose, 1 g KH_2PO_4, and 0.5 g $MgSO_4 \cdot 7H_2O$ per one liter, pH 6.8) was used for antibiotic production. *Escherichia coli* DH5α and recombinant *E. coli* cells were grown at 37°C in Luria-Bertani (LB) medium and LB medium supplemented with 50 μg/mL ampicillin, respectively. The following human pathogenic bacteria were obtained from the Korea Collection for Type Culture (KCTC) and grown on TS medium at 37°C: *E. coli* KCTC 1682, *Pseudomonas aeruginosa* KCTC 1750, *Salmonella enterica* KCTC 12456, *S. enteritidis* KCTC 12400, *S. typhimurium* KCTC 1925, *Shigella flexneri* KCTC 2008, *Shigella sonnei* KCTC 2518, *Bacillus cereus* KCTC 1012, *Listeria innocua* KCTC 3586, *L. ivanovii* KCTC 3444, *L. monocytogenes* KCTC 3569, and *Staphylococcus aureus* KCTC 162. The plant pathogenic fungi *Rhizoctonia solani*, *Pythium ultimum*, *Phytophthora capsici*, and *Fusarium oxysporum* were kindly provided by Laboratory of Phytopathology, GARES, Jinju, Korea. The plant pathogenic fungi were maintained on potato dextrose agar (PDA) and cultured at 25°C. The LB, TS, and PDA media were purchased from Difco (Becton Dickinson Co., Sparks, MD).

Isolation endophytic bacteria from YR plants. Endophytic bacteria were isolated from YR roots and leaves. Random samples (n=10) of YR were collected from GARES, Jinju, Korea. The surfaces of the leaves and roots were disinfected with 1% sodium hypochlorite for 10 min. The external portion of leaves and roots (approximately 0.5 cm from the margin) were removed with a sterile blade and the leaf and root tissues were triturated in a sterile porcelain mortar with sterile 10 mM phosphate buffer (pH 7.2). The leaf and root extracts were spread on TS agar and incubated at 28 or 37°C for 48 h. The colonies were primarily isolated and grouped by color and morphological characteristics as endophytic bacteria.

DNA extraction from bacteria. The isolated endophytic bacteria were cultured and centrifuged at 14,000 *g* for 5 min at 4°C. DNA was extracted from the pellet with the G-spin™ Genomic DNA Extraction Kit (iNtRON Biotechnology, Suwon, Korea).

Recombinant DNA techniques. PCR-amplification of 16S rDNA endophytic bacterial gene fragments was conducted using the universal primers 877F and 878R (5'CGGAGAGTTTGATCCTGG-3'; 5'-TACGGCTACC TTGTTAGCGAC-3', respectively), Super-Therm DNA polymerase (JMR, Side Cup, Kent, UK), 1.5 mM $MgCl_2$, and 2 mM dNTPs in a final volume of 50 μL. The amplification consisted of 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 90 s, followed by a final incubation at 72°C for 10 min. The anticipated product (approximately 1,500 bp) was separated with agarose gel electrophoresis of the amplified mixture and isolated using a gel extraction kit (iNtRON Biotechnology, Suwon, Korea). PCR products were directly cloned into the pGEM-T Easy vector (Promega, WI) and recombinant colonies were randomly chosen for further analysis. Standard procedures for restriction endonuclease digestion, agarose gel electro-

phoresis, purification of DNA from agarose gels, DNA ligation, and other cloning-related techniques were performed as described by Sambrook and Russel [2001]. Plasmid DNA was isolated by the Plasmid DNA Purification Kit (iNtRON Biotechnology, Suwon, Korea). Restriction enzymes and DNA modifying enzymes were purchased from Gibco-BRL (Gaithersburg, MD) and Promega (USA). Other chemicals were purchased from Sigma Chemical Co. (Saint Louis, MO).

16S rDNA sequencing and analysis. Samples were prepared for nucleotide sequencing by the dideoxy chain-termination method using the PRISM Ready Reaction Dye terminator/primer cycle sequencing kit (PerkinElmer Corp., Norwalk, CN). Sequencing was carried out with an automated DNA sequencer (Applied Biosystems, Foster City, CA). Assembly of the nucleotide sequences was performed with the DNAMAN analysis system (Lynnon Biosoft, Quebec, Canada). All reference sequences were obtained from the National Center for Biotechnology Information (NCBI) and the Ribosomal Database Project (RDP). The 16S rDNA sequences were identified using BLASTN as well as PSI-BLAST from the NCBI website [McGinnis and Madden, 2004]. Sequences were aligned using the multiple sequence alignment program CLUSTALW [Tompson *et al.*, 1994]. Gaps and ambiguities were excluded from the phylogenetic analysis, and analysis was performed using neighbor-joining methods [Saito and Nei, 1987]. Bootstrap analysis was performed 1,000 times with data re-sampling using the DNAMAN analysis system.

Extracellular hydrolytic enzyme assay. 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)), xylanase activity indicator medium (LB medium containing 0.5% (w/v) oat spelt xylan and 1.5% agar (w/v)), mannanase activity indicator medium (LB medium containing 0.5% (w/v) locust bean gum and 1.5% agar (w/v)) and linchenase activity indicator medium (LB medium containing 0.5% (w/v) linchenan and 1.5% agar (w/v)). To visualize the yellow halo zone resulting from cellulase, mannanase, xylanase and linchenase activities, plates were flooded with a 0.5% Congo Red solution for 30 min, rinsed with water, and then washed twice with 1 M NaCl. The isolates were also inoculated on PGAase activity indicator medium (LB medium containing 1.0% (w/v) polygalacturonic acid and 1.5% agar (w/v)) and amylase activity indicator medium (LB medium containing 1.0% (v/v) starch and 1.5% agar (w/v)). To visualize halo formation due to PGAase or amylase activity, the plates were flooded with 0.1% toluidine blue or potassium iodide, respectively, and washed. To determine the protease, lipase, esterase, and DNase activities, the isolates were also inoculated on protease activity indicator medium (LB medium containing 1.0% (v/v) skim milk and 1.5% agar (w/v)), lipase activity indicator medium (LB medium containing 1.0% tricaprylin (v/v) and 1.5% agar (w/v)), esterase activity indicator medium (LB medium containing 1.0% tributyrin (v/v) and 1.5% agar (w/v)), and Difco DNase activity indicator medium, respectively.

Antimicrobial activity assay. An *in vitro* bioassay was conducted to evaluate the antagonistic properties of young radish endophytic bacteria against phytopathogenic fungal species including *P. capsici, F. oxysporum, R. solani* and *P. ultimum* by the paper disk method [Cho *et al.*, 2007]. Paper disks were impregnated with 10 μL of a fungal spore suspension containing approximately 10^8 cfu/mL. The paper disks were placed on plates containing a single endophytic bacterial species, inverted, and incubated at 25°C for 48 h. Five replications were performed for all isolates. The antifungal activity was estimated by measuring the diameter of the clear zone of growth inhibition.

The agar disk diffusion technique was also used to evaluate the activity of food-borne human pathogens mentioned above against endophytic bacteria according to Barbosa *et al*. [2005]. Paper disks were impregnated with 10 μL of a bacterial suspension containing approximately 10^8 cfu/mL. The disks were placed on plates containing a single endophytic bacterial species and incubated inverted at 28°C for 48 h. The antibacterial activity was estimated by measuring the diameter of the clear zone of growth inhibition.

Results and Discussion

Isolation of culturable endophytic bacteria from the interior of YR roots and leaves. The endophytic bacterial diversity of YR plants was assessed in leaf and root samples collected from GARES. A total of 264 colonies were isolated from the interior of YR leaves and roots. Bacteria that were closely related to *Bacillus licheniformis* ATCC 145800, *Bacillus subtilis* PY79, *Stenotrophomonas* sp. EC-S105, *Myroides odoratimimus* CCUG39352^T, and *Pseudomonas* sp. PALXIL12 were detected in both plant parts (Table 1).

Samples from YR leaves contained 140 isolates representing 14 species including *Enterobacter* sp. (YRL01, 1 isolate), *B. subtilis* (YRL02 and YRL07, 13 isolates), *B. licheniformis* (YRL03, 29 isolates), *Stenotrophomonas maltophilia* (YRL04, 52 isolates), *Pseudomonas* sp. (YRL05, 2 isolates), *Stenotrophomonas* sp. (YRL06, 8 isolates), *M. odoratimimus* (YRL08, 3

Table 1. Similarity values of 16S rDNA sequences retrieved from the endophytic bacteria isolated from the interior leaf and root of a young radish

Isolates (Accession No.)	No. of isolates	Phylum	Nearest relatives[a] (Accession No.)	Similarity (%)
Leaf (140)				
YRL01 (EU373405)	1	*Proteobacteria*	*Enterobacter* sp. J11 (EU099377)	99
YRL02 (EU373407)	5	*LGCGPB*[b]	*Bacillus subtilis* C8-4 (EU257436)	99
YRL03 (EU373408)	29	*LGCGPB*	*Bacillus licheniformis* ATCC 14580 (CP000002)	98
YRL04 (EU373409)	52	*Proteobacteria*	*Stenotrophomonas maltophilia* C6 (AJ293468)	99
YRL05 (EU373411)	2	*Proteobacteria*	*Pseudomonas* sp. PALXIL12 (DQ821413)	99
YRL06 (EU373412)	8	*Proteobacteria*	*Stenotrophomonas* sp. EC-S105 (AB200253)	99
YRL07 (EU373413)	8	*LGCGPB*	*Bacillus subtilis* PY79 (EU081774)	99
YRL08 (EU373415)	3	*Bacteroidetes*	*Myroides odoratimimus* CCUG 39352^T (AJ854059)	99
YRL09 (EU373416)	1	*Proteobacteria*	*Providencia* sp. H2-3 (EF061136)	99
YRL10 (EU373417)	5	*HGCGPB*[c]	*Microbacterium* sp. PLL-3 (EU127453)	99
YRL11 (EU373418)	9	*Proteobacteria*	*Citrobacter freundii* (AF025365)	100
YRL12 (EU373420)	10	*HGCGPB*	*Brachybacterium* sp. phenol-A (DQ822566)	99
YRL13 (EU373421)	3	*LGCGPB*	*Paenibacillus polymyxa* WY110 (AY302439)	95
YRL14 (EU373423)	4	*Bacteroidetes*	*Sphingobacterium siyangensis* SY1 (EU046272)	98
Root (124)				
YRR01 (EU373425)	1	*LGCGPB*	*Bacillus licheniformis* ATCC 14580 (CP000002)	98
YRR02 (EU373426)	2	*Proteobacteria*	*Pseudomonas aeruginosa* PAL106 (DQ464061)	99
YRR03 (EU373428)	32	*LGCGPB*	*Bacillus subtilis* PY79 (EU081774)	99
YRR04 (EU373430)	23	*Proteobacteria*	*Pseudomonas* sp. 3B_8 (AY689030)	99
YRR05 (EU373431)	7	*Bacteroidetes*	*Myroides odoratimimus* CCUG 39352^T (AJ854059)	99
YRR06 (EU373433)	41	*Proteobacteria*	*Proteus vulgaris* IFAM 1731 (X07652)	99
YRR07 (EU373434)	3	*Proteobacteria*	*Pseudomonas* sp. PALXIL12 (DQ821413)	99
YRR08 (EU373435)	5	*HGCGPB*	*Microbacterium* sp. VKM Ac-1781 (AB042072)	97
YRR09 (EU373437)	3	*Proteobacteria*	*Stenotrophomonas* sp. EC-S105 (AB200253)	99
YRR10 (EU373438)	3	*LGCGPB*	*Bacillus subtilis* 168 (Z99104)	99
YRR11 (EU373439)	4	*Proteobacteria*	*Agrobacterium tumefaciens* DMS 30105 (M11223)	99

[a]Range of 16S rDNA genes sequence is similarity values between endophytic bacteria and type strain.
[b]LGCGPB: low G+C Gram-positive bacteria
[c]HGCGPB: high G+C Gram-positive bacteria

isolates), *Providencia* sp. (YRL09, 1 isolate), *Microbacterium* sp. (YRL10, 10 isolates), *Citrobacter freundii* (YRL11, 9 isolates), *Brachybacterium* sp. (YRL12, 10 isolates), *Paenibacillus polymyxa* (YRL13, 3 isolates), and *Sphingobacterium siyangensis* (YRL14, 4 isolates). Each of the rDNA sequences was 95 to 100% similar to those found in the databases (Table 1).

The YR root samples contained 124 isolates from 11 species. The clones had 97 to 100% sequence similarity with culturable isolates: YRR01 (1 isolate) with *B. licheniformis,* YRR02 (2 isolates) with *P. aeruginosa,* YRR03 (32 isolates) and YRR10 (3 isolates) with *B. subtilis,* YRR04 and YRR07 (26 isolates) with *Pseudomonas* sp., YRR05 (7 isolates) with *M. odoratimimus*, YRR06 (41 isolates) with *Proteus vulgaris*, YRR08 (5 isolates) with *Microbacterium* sp., YRR09 (3 isolates) with *Stenotrophomonas* sp., and YRR11 (4 isolates) with *Agrobacterium tumefaciens* (Table 1).

McInroy and Klopper [1995] isolated 34 endophytic genera from 1,029 isolates in the interior of roots and stems of cotton and sweet corn. Lilley *et al.* [1996] found 23 genera in only 114 endophytic isolates from sugar beet. Germida *et al.* [1998] isolated 18 endophytic bacterial genera in 220 isolates from the root tissues of three field-grown canolas. Sturz *et al.* [1997] found that the endophytic bacterial population exhibited differences depending on the host plant species. Siciliano and Germida [1999] also identified 27 bacterial genera among 1,100 bacterial isolates from the interior roots of three canola cultivars. In previous, Cho *et al.* [2007] identified 13 bacterial genera among 63 bacterial isolates in the interior roots of ginseng from three areas. Thus, it appears that the diversity of endophytic communities varies significantly among plant species.

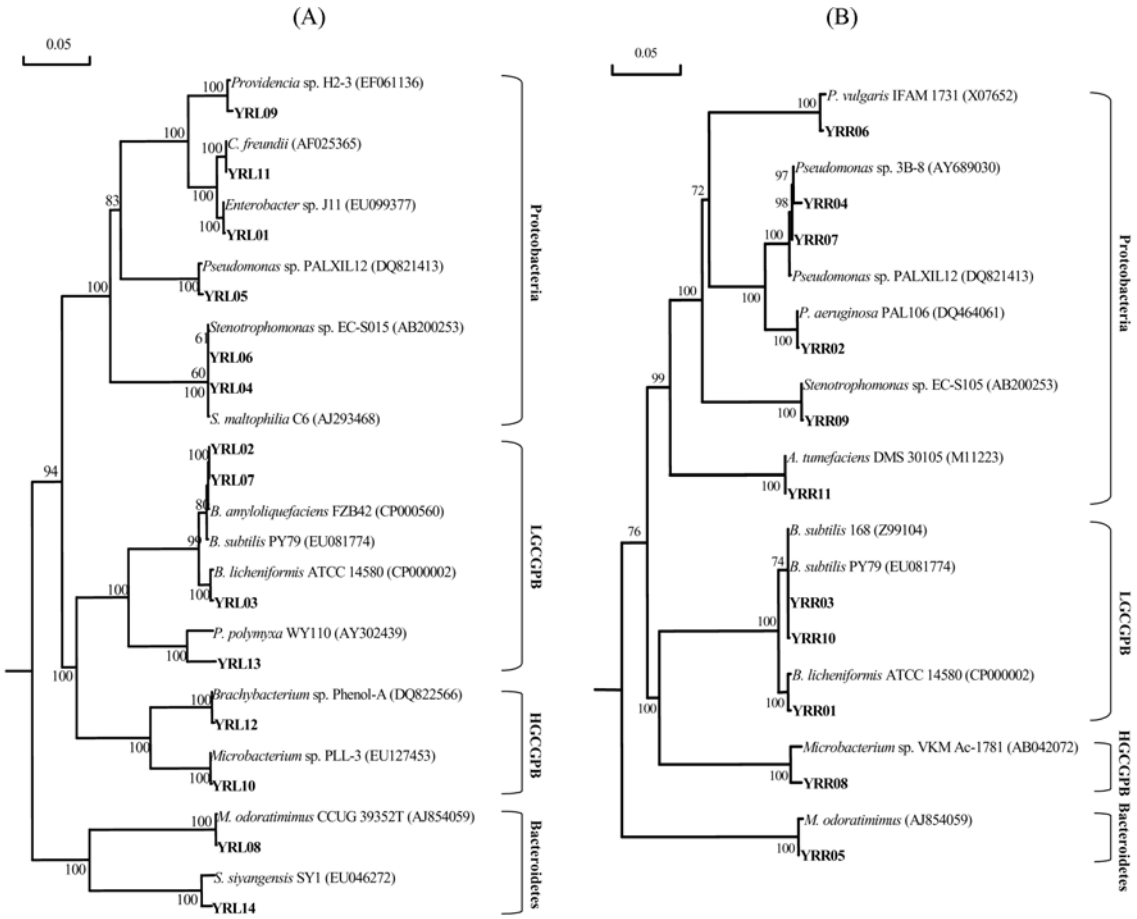

Fig. 1. Phylogeneitc placement of 16S rDNA sequence from the endophytic bacteria of the young radish (YR) leaf (A) and root (B). Numbers above each node are confidence levels (%) generated from 1,000 bootsrap trees. The scale bar is in fixed nucleotide substations per sequence position.

Phylogenetic tree analysis of 16S rDNA sequences from YR endophytic bacteria. Phylogenetic analysis of 16S rDNA genes revealed the presence of endophytic bacteria belonging to the *HGCGPB* (high G+C gram positive bacteria), *LGCGPB* (low G+C gram positive bacteria), *Proteobacteria*, and *Bacteroidetes* groups in the leaves and roots of YR (Fig. 1).

The phylogenetic analysis of the endophytic bacteria from the YR leaves is shown in Fig. 1A. The endophytic bacteria grouped into four clusters based on the 16S rDNA sequences: *HGCGPB*, *LGCGPB*, *Proteobacteria*, and *Bacteroidetes*. The *HGCGPB* cluster included *Brachybacterium* sp. (YRL12) and *Microbacterium* sp. (YRL10). The *LGCGPB* cluster included *B. licheniformis* (YRL03), *B. subtilis* (YRL07), *B. subtilis* (YRL02), and *P. polymyxa* (YRL13). The *Proteobacteria* cluster included *Enterobacter* sp. (YRL01), *Pseudomonas* sp. (YRL05), *Stenotrophomonas* sp. (YRL06), *Providencia* sp. (YRL09), *C. freundii* (YRL11), and *S. maltophilia* (YRL04). The *Bacteroidetes* cluster contained *M. odoratimimus* (YRL08) and *S. siyangensis* (YRL14).

Figure 1B shows the phylogenetic analysis of endophytic bacteria from the YR roots. The 16S rDNA sequences divided the samples into four clusters: *HGCGPB, LGCGPB, Proteobacteria*, and *Bacteroidetes*. The *HGCGPB* cluster only had *Microbacterium* sp. (YRR08) while the *LGCGPB* group consisted of *B. subtilis* (YRR03 and YRR10) and *B. licheniformis* (HNL09). The *Proteobacteria* cluster included *Pseudomonas* sp. (YRR04 and YRR07), *P. vulgaris* (YRR06), *P. aeruginosa* (YRR02), and *Stenotrophomonas* sp. (YRR09). Finally, the *Bacteroidetes* cluster only included *M. odoratimimus* (YRR05).

Proteobacteria were the predominant species isolated from root (52.1%) and leaf (61.3%) samples (Fig. 2). Bacteria belonging to *Pseudomonas* and *Bacillus* genera are easy to culture and cultivation-dependent studies have identified them as frequently occurring endophytes [Seghers *et al.,* 2004; Rosenblueth and Martínez-Romero, 2006; Cho *et al.,* 2007]. Moreover, the *HGCGPB* and *Bacteroidetes* populations have been isolated from wheat, potato, maize, and other species [Coombs and Franco,

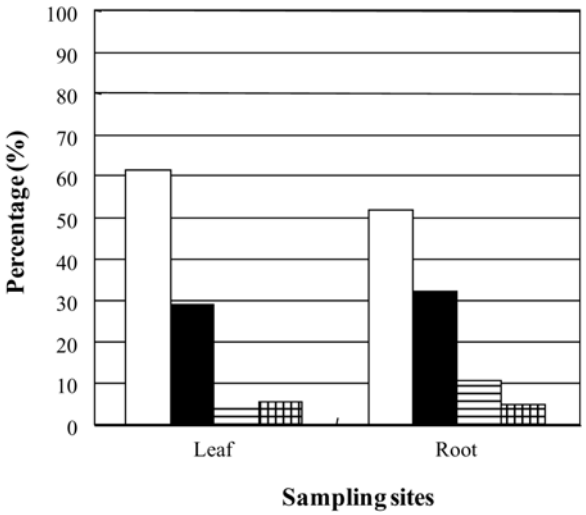

Fig. 2. Distribution of the 16S rDNA sequences of isolates in each of the young radish (YR) leaf and root samples. Numbers in square brackets was given the total number of the corresponding isolates in that sample sites. Percentage of microcosm in each of two sample sites was shown. *Proteobacteria* (□), *LGCGPB* (■), *HGCGPB* (▤), *Bacteroidetes* (▦).

2003; Conn and Franco, 2004; Rosenblueth and Martínez-Romero, 2006]. In previous, Ulrich *et al.* [2008] studied endophytic bacteria of the poplar grown under field conditions and found a high level of phylogenetic diversity in endophytic bacteria with a total of 53 taxa at the genus level, including *Proteobacteria*, *HGCGPB*, *LGCGPB*, and *Bacteroidetes*.

Identification of extracellular enzymatic activity from YR endophytic bacteria. The bacteria isolated from the YR were evaluated for the presence of active hydrolytic enzymes including amylase, cellulase, xylanase, mannase, PGAase, DNase, protease, lipase, and esterase (Table 2). *B. subtilis* YRL02, YRL07, YRR03, and YRR10 were positive for all enzymes tested. *B. licheniformis* YRL02 and YRR01 exhibited all enzyme activities with the exceptions of amylase, lipase, and esterase. All of the isolates tested positive for DNase activity except for *Pseudomonas* sp. YRL05 and *Microbacterium* sp YRL10. Endophytic bacteria of the YR root showed higher cellulase, DNase and protease activities than bacteria isolated from YR leaves. Endophytic bacteria of the YR leaves showed the higher pectinase and lipase activities than the population from the roots (Table 2).

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 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]. Endophytic bacteria likely have a signaling mechanism (quorum-sensing) that specifically regulates the amount and timing of enzyme production. Interestingly, plants can perceive these signals from the bacteria and control quorum-regulated bacterial responses [Mathesius *et al.,* 2003; Bauer and Mathesius, 2004]. It would be interesting to determine if endophytes produce quorum-sensing molecules inside the plants and to study their effects. There could be an exchange of signal molecules among microorganisms inside the plant and between bacteria and the host, though this has not been reported.

Endophytes are considered to be a new source of genes, proteins, and biochemical compounds that may be useful in various industrial fields. Several hydrolysis enzymes previously studied from endophytic bacteria, such as cellulase from *Bacillus pumilus* [Lima *et al.* 2005], α-amylase from *Nocardiopsis* sp. [Stamford *et al.* 2001], glucoamylas from *Streptosporangium* sp. [Stamford *et al.* 2002] and levansucrase from *Acetobacter diazotrophicus* [Arrieta *et al.,* 1996; Menendez *et al.* 2002]. Also, we previously reported the complete nucleotide sequence of the multifunctional endoglucanase gene, referred to as *cel*44C-*man*26A from *P. polymyxa* GS01 [Cho *et al.* 2006]. Similarly, YR endophytic bacteria exhibited different enzymatic activities and thus can be a new source for commercial enzyme production.

Estimation of the antimicrobial activity of endophytic bacteria isolated from YR against plant pathogenic fungi. Antifungal activity of endophytic bacteria isolated from YR was evaluated against four plant pathogenic fungi including *R. solani*, *F. oxysporum*, *P. ultimum,* and *P. capsici* (Table 3).

Among all of the isolates from YR roots and leaves, *Providencia* sp. (YRL09), *Pseudomonas* sp. (YRR04), and *B. subtilis* (YRR10) exhibited broad spectrums of antifungal activity in *in vitro* tests. *B. subtilis* (YRR10) had especially strong antifungal activity against all tested plant pathogenic fungi. *B. amyloliquefaciens* (YRL02), *Pseudomonas* sp. (YRL05), *B. subtilis* (YRL02, YRL07 and YRR03), and *M. odoratimimus* (YRR05) had antifungal activity against *P. capsici*, *F. oxysporum,* and *R. solani* whereas *P. polymyxa* YRL13 exhibited antifungal activity against *P. capsici, P. ultimum*, and *R. solani.* In

Table 2. Identification of the various extracellular enzyme activities from the endophytic bacteria of the interior leaf and root of a young radish

Isolates	Enzyme activity[a]								
	Amylase	Cellulase	Xylanase	Mannase	PGAase	DNase	Protease	Lipase	Esterase
Leaf									
Enterobacter sp. YRL01	-	-	-	-	-	w	-	-	-
Bacillus subtilis YRL02	++	+	++	+++	+	w	+++	-	+
Bacillus licheniformis YRL03	-	++	+	+	+	w	+	-	-
Stenotrophomonas maltophilia YRL04	-	++	-	-	-	w	+	-	-
Pseudomonas sp. YRL05	-	-	-	-	-	-	+++	-	-
Stenotrophomonas sp. YRL06	-	+	-	-	-	++	+	+	-
Bacillus subtilis YRL07	++	+	++	++	+	+	+	-	+
Myroides odoratimimus YRL08	+	-	-	-	-	+	-	-	-
Providencia sp. YRL09	-	-	-	-	-	+	-	-	-
Microbacterium sp. YRL10	++	-	-	-	-	-	-	-	-
Citrobacter freundii YRL11	-	-	-	-	-	+	-	-	-
Brachybacterium sp. YRL12	+	-	-	-	-	+	-	-	-
Paenibacillus polymyxa YRL13	++	-	-	+++	-	+	-	+	-
Sphingobacterium siyangensis YRL14	-	-	-	-	-	w	-	-	-
Root									
Bacillus licheniformis YRR01	-	++	+	+	+	+	+	-	-
Pseudomonas aeruginosa YRR02	-	-	-	-	-	++	-	+	-
Bacillus subtilis YRR03	++	+	+++	+++	+	w	+	-	++
Pseudomonas sp. YRR04	-	-	-	-	+	w	-	-	-
Myroides odoratimimus YRR05	-	-	+	-	-	++	-	+	-
Proteus vulgaris YRR06	-	-	-	-	+	+	-	+	-
Pseudomonas sp. YRR07	-	-	-	-	-	+	-	+	-
Microbacterium sp. YRR08	-	w	-	-	+	+	+	+	-
Stenotrophomonas sp. YRR09	-	+	-	-	-	++	+	+	-
Bacillus subtilis YRR10	+	+	++	+	+	++	+	-	+
Agrobacterium tumefaciens YRR11	-	-	-	-	+	++	-	+	-

[a] Size of halos formed around bacterial colonies on agar media. Symbols: -, implies no halo zone indicates no enzyme activity; w, implies <2 mm diameter of the halo zone indicates weak enzyme activity; +, implies 2 to 4 mm diameter of the halo zone indicates lower enzyme activity; ++, implies 4 to 6 mm diameter of the halo zone indicates medium enzyme activity; +++, implies >6 mm diameter of the halo zone indicates higher enzyme activity, respectively.

root samples, endophytic bacteria with antifungal activity against *R. solani* were most common, but in leaf samples bacteria with activity against *F. oxysporum* were predominant (Table 3).

The potential use of endophytic bacteria for biocontrol agents to protect crops from fungal diseases has been investigated in only a limited number of plant species. This topic is of special interest because the same bacterium could promote growth of the host as well as provide protection against pathogens. There is some evidence that endophytic bacteria such as *Pseudomonas* and *Bacillus* can provide protective antifungal effects on crops including cotton, potatoes, tomatoes, balloon flowers, and ginseng [Cho *et al.,* 2002; Berg *et al.,* 2005; Cho *et al.,* 2007; Enya *et al.,* 2007]. Among the studied endophytic bacteria, *Bacillus* sp. is stable in the soil, and has a great potential as a biocontrol agent due to its spore stability, ease of handling and the production of spore-specific antifungal lipopeptides. The endophytic *Bacillus* sp. CY22 recovered from the interior of a balloon flower root was effective as a biocontrol agent against plant pathogenic fungi including *R. solani* [Cho *et al.,* 2002]. In previous, Cho *et al.* [2007] suggested that three isolated endophytic bacteria from ginseng (*P. polymyxa* GS01, *Bacillus* sp. GS07, and *Pseudomonas poae* JA01) had potential as biocontrol agents against plant pathogenic fungi. Moreover, it has been recognized recently that endophytic bacteria play an important role in resistance to disease and that signals exist to mediate cross talk between the endophytes and their host. One important factor postulated for the

Table 3. *In vitro* inhibitory activity[a] against the plant pathogenic fungi by young radish leaf and root endophytic bacteria

Isolates	Plant pathogenic fungi			
	Phytophyhora capsii	*Fusarium oxysporum*	*Rhizoctonia solani*	*Phythium ultimun*
Leaf				
Enterobacter sp. YRL01	-	-	-	-
Bacillus subtilis YRL02	8.8	12.0	12.5	-
Bacillus licheniformis YRL03	-	-	-	-
Stenotrophomonas maltophilia YRL04	-	-	-	-
Pseudomonas sp. YRL05	9.2	8.8	9.6	-
Stenotrophomonas sp. YRL06	-	9.5	-	-
Bacillus subtilis YRL07	9.8	9.2	11.6	-
Myroides odoratimimus YRL08	8.4	11.8	8.6	9.2
Providencia sp. YRL09	9.2	8.4	9.4	10.8
Microbacterium sp. YRL10	-	-	-	-
Citrobacter freundii YRL11	-	8.2	-	13.6
Brachybacterium sp. YRL12	12.1	-	8.9	-
Paenibacillus polymyxa YRL13	10.6	-	10.0	12.2
Sphingobacterium siyangensis YRL14	-	-	-	-
Root				
Bacillus licheniformis YRR01	-	-	-	-
Pseudomonas aeruginosa YRR02	-	-	-	8.6
Bacillus subtilis YRR03	9.4	8.6	11.2	-
Pseudomonas sp. YRR04	9.2	9.6	10.0	8.8
Myroides odoratimimus YRR05	8.4	8.9	8.4	-
Proteus vulgaris YRR06	-	-	-	-
Pseudomonas sp. YRR07	-	-	-	-
Microbacterium sp. YRR08	14.8	8.9	9.3	-
Stenotrophomonas sp. YRR09	-	-	-	9.8
Bacillus subtilis YRR10	15.2	8.6	11.6	9.6
Agrobacterium tumefaciens YRR11	-	-	-	8.8

[a]The antifungal activity was estimated by measuring the diameter of the clear zone (including paper disks, 8 mm diameter) of growth inhibition.

optimal performance of an introduced endophytic microbe is the relationship between the plant genotype and effective colonization [Sturz and Nowak, 2000].

Estimation of antimicrobial activity against human pathogenic bacteria of endophytic bacteria isolated from YR. Antibacterial activity of endophytic bacteria isolated from YR was evaluated against several human pathogenic bacteria (Table 4). Among the bacteria isolated from leaf samples, *Enterobacter* sp. YRL01 showed the strongest antibacterial activity against food borne pathogens including *E. coli*, *S. enteritidis*, *S. typhimurium*, *S. flexneri*, *S. sonnei*, *P. aeruginosa*, *B. cereus*, and *L. innocua.* Secondly, *B. subtilis* YRL02 also exhibited antibacterial activity against *S. enteritidis*, *S. flexneri*, *S. sonnei*, *P. aeruginosa*, and *L. innocua.* Among the endophytic bacteria isolated from root samples, *B. subtilis* YRR10 showed the strongest activity against *S. enterica*, *S. sonnei* and *L. monocytogenes*.

Endophytic bacteria produce antibiotics, which can act against human pathogenic bacteria. Thus, endophytes can be a good source for the industrial production of antibiotics. Strobel *et al.* [2004] suggested that endophytes can aid their host plant by producing a plethora of substances that provide protection and ultimately help the plant to survive. Finally, after these compounds are isolated and characterized they may have a potential use in modern medicine, agriculture, or industry. However, endophytes are closely related to human pathogens and some can be either human or opportunistic human pathogens. Examples of this include endophytic *Salmonella* strains, which have caused outbreaks and constitute a health risk for consumers of raw fruits and vegetables [Guo *et al.* 2002]. In the present study, two human pathogenic bacteria, *P. aeruginosa* YRR02 and *P. vulgaris* YRR06, were found in endophytic bacterial populations of young radish roots. Iniguez *et al.* [2005]

Table 4. *In vitro* inhibitory activity[a] against the human pathogenic bacteria by young radish leaf and root endophytic bacteria

Isolates	Human pathogenic bacteria[b]											
	Eci	*Sea*	*Ses*	*Stm*	*Sfi*	*Ssi*	*Paa*	*Bcs*	*Lia*	*Lii*	*Lms*	*Sas*
Leaf												
Enterobacter sp. YRL01	14.6	-	9.2	13.8	12.7	8.6	8.4	11.8	11.4	-	-	-
Bacillus subtilis YRL02	-	-	16.2	-	15.4	9.2	11.6	-	15.6	-	-	-
Bacillus licheniformis YRL03	-	-	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas maltophilia YRL04	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas sp. YRL05	-	12.4	-	-	-	-	-	-	-	-	9.3	-
Stenotrophomonas sp. YRL06	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis YRL07	-	-	-	-	-	-	-	-	-	-	-	-
Myroides odoratimimus YRL08	-	-	-	-	-	-	-	-	-	-	-	8.6
Providencia sp. YRL09	-	-	-	-	-	-	-	-	-	-	-	-
Microbacterium sp. YRL10	-	-	-	-	-	-	-	-	-	-	-	-
Citrobacter freundii YRL11	-	-	-	-	-	-	-	-	-	-	-	-
Brachybacterium sp. YRL12	-	-	-	-	-	-	-	-	-	-	-	-
Paenibacillus polymyxa YRL13	-	-	-	-	-	9.2	-	-	-	-	-	-
Sphingobacterium siyangensis YRL14	-	-	8.2	-	-	8.8	-	-	-	-	-	9.5
Root												
Bacillus licheniformis YRR01	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa YRR02	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis YRR03	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas sp. YRR04	-	12.6	-	-	-	-	-	-	-	-	12.3	-
Myroides odoratimimus YRR05	-	-	-	-	-	-	-	-	-	-	-	-
roteus vulgaris YRR06	-	9.6	-	-	-	-	-	-	-	-	-	-
Pseudomonas sp. YRR07	-	8.7	-	-	-	-	-	-	-	-	-	-
Microbacterium sp. YRR08	-	9.2	-	-	-	-	-	-	-	-	-	-
Stenotrophomonas sp. YRR09	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis YRR10	-	8.6	-	-	-	8.8	-	-	-	-	11.6	-
Agrobacterium tumefaciens YRR11	-	-	-	-	-	-	-	-	-	-	-	-

[a]The antibacterial activity was estimated by measuring the diameter of the clear zone (including paper disks, 8 mm diameter) of growth inhibition.

[b]Human pathogenic bacteria: *Eci, Escherichia coli* KCTC 1682; *Paa*, *Pseudomonas aeruginosa* KCTC 1750; *Sea*, *Salmonella enterica* KCTC 12456; *Ses*, *Salmonella enteritids* KCTC 12400; *Stm*, *Salmonella typhimurium* KCTC 1925; *Sfi*, *Shigella flexineri* KCTC 2008; *Ssi*, *Shigella sonnei* KCTC 2518; *Bcs*, *Bacillus cereus* KCTC 1012; *Lia*, *Listeria innocula* KCTC 3586; *Lii*, *L. ivanovii* KCTC 3444; *Lms*, *Listeria monocytogenes* KCTC 3569; *Sas*, *Staphylococcus aureus* KCTC 1621.

suggested approaches to reduce the contamination of raw vegetables and proposed strategies to increase the number of safe growth-promoting bacteria in plants. Parke and Gurian-Sherman [2001] stated, "It is not coincidental perhaps that many of the most effective biocontrol agents (*Stenotrophomonas maltophilia*, *Pantoea agglomerans*, and *Burkholderia cepacia* etc.) of plant diseases are also opportunistic human pathogens. These are fiercely competitive for nutrients and may produce antimicrobial metabolites and may themselves be resistant to multiple antibiotics." Rosenblueth and Martínez-Romero [2006] explained that human pathogens may not killed by disinfection procedures that eliminate superficially occurring bacteria. In addition, the plant defense mechanisms imposed on endophytic bacteria may render them resistant to human defense responses as well. Thus, it is important to characterize the antibacterial activity of beneficial endophytic bacteria to optimize their potential for the elimination for human pathogenic bacteria.

In conclusion, diverse endophytic bacteria were obtained from YR root and leaf samples from GARES, Jinju, Korea. In this study 264 different endophytic bacteria belonging to 20 bacterial genera were isolated. *B. subtilis* YRL02, YRL07, YRR03, and YRR10 had the strongest extracellular enzymatic activity. In addition, the isolated endophytic *Enterobacter* sp. YRL01 and *B. subtilis*

YRL02 were able to inhibit the growth of human pathogenic bacteria. In particular, *B. subtilis* YRR10 inhibited the growth of plant pathogenic fungi. By further screening of the antimicrobial activity of endophytic bacterial strains of the YR against human and plant pathogens may expand the application of these antimicrobial compounds as biocontrol agents. Also, further research at the molecular level must be conducted to study the ecology and interactions of endophytes.

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References

Al-Mallah MK, Davey MR, and Cooking EC (1987) Enzymatic treatment of clover root hairs removes a barrier to *Rhizobium*-host specificity. *Biotechnology* **5**, 1319-1322.

An DJ, Lew KC, and Lee KP (1999) Effects of adipic acid and storage temperature on extending the shelf life of *kimchi*. *Food Sci Biotechnol* **8**, 78-82.

Araújo WL, Marcon J, Maccheroni W, Jr van Elsas JD, van Vuurde JWL, and Azevedo JL (2002) Diversity of endophytic bacterial populations and their interaction with *Xylella fatidiosa* in citrus plants. *Appl Environ Microbiol* **68**, 4906–4914.

Arrieta J, Hernandez L, Coego A, Suarez V, Balmori E, Menendez C, Petit-Glatron MF, Chambert R, and Selman-Housein G (1996) Molecular characterization of the levansucrase gene from the endophytic sugarcane bacterium *Acetobacter diazotrophicus* SRT4. *Microbiology* **142**, 1077-1085.

Barbosa TM, Serra CR, Ragione RML, Woodward MJ, and Henriques AO (2005) Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Appl Environ Microbiol* **71**, 968-978.

Bauer WD and Mathesius U (2004) Plant responses to bacterial quorum sensing singals. *Curr Opin Plant Biol* **7**, 429-433.

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, and Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. *FEMS Microbiol Ecol* **51**, 215-229.

Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Kim H, and Yun HD (2006) A *cel*44C-*man*26A gene of endophytic *Paenibacillus polymyxa* GS01 has multi-glycosyl hydrolases in two catalytic domains. *Appl Microbiol Biotechnol* **73**, 618-630.

Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Lim YP, Kim H, and Yun HD (2007) Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. *Microbial Ecol* **54**, 341-351.

Cho SJ, Park SR, Kim MK, Lim WJ, Ryu SK, An CL, Hong SY, Lee YH, Jeong SG, Cho YU, and Yun HD (2002) Endophytic *Bacillus* sp. isolated from the interior of ballon flower root. *Biosci Biotechnol Biochem* **66**, 1270-1275.

Conn VM and Franco CMM (2004) Effect of microbial inoculants on the indigenous actinobacterial endophyte population in the roots of wheat as determined by terminal restriction fragment length polymorphism. *Appl Environ Microbiol* **70**, 6407-6413.

Coombs JT and Franco CMM (2003) Isolation and identification of *Actinobacteria* from surface-sterilized wheat roots. *Appl Environ Microbiol* **69**, 5603-5608.

Elvira-Recuenco M and van Vuurde JWL (2000) Natural incidence of endophytic bacteria in pea cultivars under field conditions. *Can J Microbiol* **46**, 1036-1041.

Enya J, Koitabshi M, Shinohara H, Yoshida S, Tsukiboshi T, Negishi H, Suyama K, and Tsushima S (2007) Phylogenetic diversities of dominant culturable *Bacillus*, *Pseudomonas* and *Pantoea* species on tomato leaves and their possibility as biological control agents. *J Phytopathol* **155**, 446-453.

Garbeva P, Overheek van LS, Vuurde van JWI, and van Elsas JD (2001) Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial Ecol* **41**, 360-383.

Germida JJ, Siciliano SD, Freitas de JR, and Seib AM (1998) Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *FEMS Microbiol Ecol* **26**, 43-50.

Granér G, Persson P, Meijer J, and Alström S (2003) A study on microbial diversity in different cultivars of *Brassica napus* in relation to its wilt pathogen, *Verticillium longisporum. FEMS Microbiol Lett* **224**, 269-276.

Guo X, van Iersel MW, Chen J, Brackett RE, and Beuchat LR (2002) Evidence of association of salmonellae with tomato plants grown hydroponically in inoculated solution. *Appl Environ Microbiol* **68**, 3639-3643.

Hallmann J, Quadt-Hallmann A, Mahaffee WF, and Kloepper JW (1997) Bacterial endophytes in agricoltural crops. *Can J Microbiol* **43**, 895-914.

Iniguez AL, Dong Y, Carter HD, Ahmer BMM, Stone JM, and Triplett EW (2005) Requlation of enteric endophytic bacterial colonization by plant defense. *Mol Plant-Microbe Interact* **18**, 169-178.

Lilley AK, Fry JC, Bailey MJ, and Day MJ (1996) Comparison of aerobic heterotropic taxa isolated from four root domains of mature sugar beet (*Beta vulgaris). FEMS Microbiol Ecol* **21**, 231-242.

Lima AOS, Quecine MC, Fungaro MHP, Andreote FD, Jr Maccheroni W, Araújo WL, Silva-Filho MC, Pizzirani-Kleiner AA, and Azevedo JL (2005) Molecular characterization of a β-1,4-endoglucanase from an endophytic *Bacillus pumilus* strain. *Appl Microbiol*

Biotechnol **68**, 57-65.

Mathesius U, Mulders S, Gao M, Teplitski M, Caetano-Anolles G, Rolfe BG, and Baurer WD (2003) Extensive and specific response of a eukaryotes to bacterial quorum-sensing signals. *Proc Natl Acad Sci USA* **100**, 1444-1449.

McGinnis S and Madden TL (2004) BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Res* **32**, 20-25.

McInroy JA and Kloepper JW (1995) Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant Soil* **173**, 337-342.

Menendez C, Hemandez L, Selman G., Mendoza MF, Hevia P, Sotolongo M, and Arrieta JG (2002) Molecular cloning and expression in *Escherichia coli* of an exo-levanase gene from the endophytic bacterium *Gluconacetobacter diazotrophicus* SRT4. *Curr Microbiol* **45**, 5-12.

Mocali S, Bertelli E, Cello FD, Mengoni A, Sfalanga A, Viliani F, Caciotti A, Tegli S, Surico G, and Fani R (2003) Fluctuation of bacteria isolated from elm tissues during different seasons and from different plant organs. *Res Microbiol* **154**, 105-114.

Mundt JO and Hinkle JO (1976) Bacteria within ovules and seeds. *Appl Environ Microbiol* **32**, 694-698.

Parke JL and Gurian-Sherman D (2001) Diversity of the *Burkholderia cepacia* complex and implications for risk assessment of biological control strains. *Annu Rev Phytopathol* **39**, 225-258.

Quadt-Hallmann A, Benhamou AN, and Kleopper JW (1997) Bacterial endophytes in cotton: Mechanisms of entering the plant. *Can J Microbiol* **43**, 577-582.

Reinhold-Hurek B and Hurek T (1998) Life in grasses: Diazotrophic endophytes. *Trend Microbiol* **6**, 139-144.

Reiter B, Pfeifer U, Schwab H, and Sessitsch A (2002) Response of endophytic bacterial communities in potato plants to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl Environ Microbiol* **68**, 2261-2268.

Rosenblueth M and Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. *Mol Plant-Microbe Interat* **19**, 827-837.

Saito N and Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Sakiyama CCH, Paula EM, Pereira PC, Borges AC, and Silva DO (2001) Characterization of pectin lyase produced by an endophytic strain isolated from coffee cherries. *Lett Appl Microbiol* **33**, 117-121.

Sambrook J and Russel DW (2001) In *Molecular Cloning*: A Laboratory Manual, (3th ed.). Cold Spring Harbor Laboratory Press, New York, NY, U.S.A.

Seghers D, Wittebolle L, Top EM, Verstraete W, and Siciliano SD (2004) Impact of agricultural practice on the *Zea mays* L. endophytic community. *Appl Environ Microbiol* **70**, 1475-1482.

Siciliano SD and Germida JJ (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. *FEMS Microbiol Ecol* **29**, 263-272.

Stamford TL, Stamfod NP, Coelho LC, and Araujo JM (2001) Production and characterization of a thermostable alpha-amylase from *Nocardiopsis* sp. endophyte of yam bean. *Bioresour Technol* **76**, 137-141.

Stamford TL, Stamfod NP, Coelho LC, and Araujo JM (2002) Production and characterization of a thermostable glucoamylase from *Streptosporangium* sp. endophyte of maize leaves. *Bioresour Technol* **83**, 105-109.

Strobel G and Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* **67**, 491-502.

Strobel G, Daisy B, Castillo U, and Harper J (2004) Natural products from endophytic microorganisms. *J Nat Prod* **67**, 257-68.

Sturz AV, Chrssistie BR, Matheson BG, and Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol Fertil Soils* **25**, 13–19.

Sturz AV and Nowak J (2000) Endophytic communities of rhizobacter and the strategies required to create yield enhancing associations with crops. *Appl Soil Ecol* **15**, 183-190.

Tompson JD, Higgins DG, and Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673-4680.

Ulrich K, Ulrich A, and Ewald D (2008) Diversity of endophytic bacterial communities in poplar grown under field conditions. *FEMS Microbiol Ecol* **63**, 169-80.

Verma SC, Ladha JK, and Tripathi AK (2001) Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *J Biotechnol* **81**, 127-141.