Journal of Microbiology (2016) Vol. 54, No. 5, pp. 353–363
DOI 10.1007/s12275-016-5641-7
eISSN 1976-3794
pISSN 1225-8873

Diversity of indigenous endophytic bacteria associated with the roots of Chinese cabbage (*Brassica campestris* L.) cultivars and their antagonism towards pathogens[§]

Md. Azizul Haque[1,3], Han Dae Yun[2], and Kye Man Cho[1*]

[1]*Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 660-758, Republic of Korea*
[2]*Research Institute of Life Sciences, Gyeongsang National University, Jinju 660-701, Republic of Korea*
[3]*Department of Biochemistry & Molecular Biology, Hajee Mohammad Danesh Science & Technology University, Dinajpur 5200, Bangladesh*

(Received Dec 28, 2015 / Revised Apr 4, 2016 / Accepted Apr 6, 2016)

The study aimed to reveal the diversity of endophytic bacteria in the roots of Chinese cabbage (CC) cultivated in two areas in Korea, namely, Seosang-gun (SS) and Haenam-gun (HN), and also in a transgenic plant (TP) from the laboratory. A total of 653 colonies were isolated from the interior of CC roots, comprising 118, 302, and 233 isolates from SS, HN, and TP samples, respectively. Based on 16S rRNA gene sequence analysis, the isolates belonged to four major phylogenetic groups: high-G+C Gram-positive bacteria (HGC-GPB), low-G+C Gram-positive bacteria (LGC-GPB), *Proteobacteria*, and *Bacteriodetes*. The most dominant groups in the roots of the SS, HN, and TP cultivars were LGC-GPB (48.3%), *Proteobacteria* (50.2%), and HGC-GPB (38.2%), respectively. Importantly, most of the isolates that produced cell-wall-degrading enzymes belonged to the genus *Bacillus*. *Bacillus* sp. (HNR03, TPR06), *Bacillus pumilus* (SSR07, HNR11, TPR07), and *Bacillus subtilis* (TPR03) showed high antagonism against the tested food-borne pathogenic bacteria. In addition, *Bacillus* sp. (HNR03, TPR06), *Bacillus pumilus* (SSR07, HNR11, HNR17, TPR11), *Microbacterium oxidans* (SSR09, TPR04), *Bacillus cereus* HNR10, *Pseudomonas* sp. HNR13, and *Bacillus subtilis* (TPR02, TPR03) showed strong antagonistic activity against the fungi *Phythium ultimum*, *Phytophthora capsici*, *Fusarium oxysporum*, and *Rhizoctonia solani*. The endophytes isolated from the TP cultivar showed the strongest antagonistic reactions against pathogens. This study is the first report on endophytic bacteria from Chinese cabbage roots.

Keywords: Chinese cabbage root, endophytic bacteria, phylogene, antimicrobial activity

Introduction

Chinese cabbage (*Brassica campestris* L.) has been one of the most important vegetable crops in eastern Asia for many centuries. This cabbage is a major raw material of Korean traditionally fermented *kimchi* (Park, 1995). The consumption of Chinese cabbage (CC) *kimchi* may improve health because of its anti-carcinogenic secondary metabolites (Haque *et al.*, 2015). Thus, the popularity of Chinese cabbage *kimchi* and its cultivation are increasing globally. However, among major factors limiting Chinese cabbage yield is its susceptibility to a variety of pathogenic fungi. *Fusarium* wilt, induced by pathogenic strains of *Fusarium oxysporium*, is a serious soil-borne disease in many crops of economic importance including Chinese cabbage (Khastini *et al.*, 2012). *Fusarium oxysporium* invades roots and cause wilt diseases through colonization in the xylem tissue of host plants (Ohike *et al.*, 2013).

Plants provide a nutrient-rich niche for the growth and development of microorganisms, particularly endophytic bacteria (Strobel and Daisy, 2003; de Melo Pereira *et al.*, 2012). Endophytic bacteria are known to be involved in plant nutrition (Dalton *et al.*, 2004), morphogenesis (Fukui *et al.*, 2014), stress or defense responses (Li *et al.*, 2012), against invading fungal phytopathogens (Li *et al.*, 2010; Prieto *et al.*, 2011; Luo *et al.*, 2013; Pathak and Keharia, 2013), and growth promotion (Sessitsch *et al.*, 2004; Andrade *et al.*, 2014; Falcao *et al.*, 2014; Khan *et al.*, 2014). Bacterial endophytes penetrate into plant roots, stems or leaves using enzymes capable of hydrolyzing extracellular cell walls (Cho *et al.*, 2007; Islam *et al.*, 2010). However, the extracellular hydrolytic enzymes of bacterial endophytes are involved in the suppression of pathogenic fungi by biocontrol agents (Ordentlich *et al.*, 1988; Luo *et al.*, 2013). Therefore, the use of antagonistic bacteria as biocontrol agents is convenient, for it can be directly applied to seeds when planting or mixed into soils (Marimuthu *et al.*, 2013). The use of bacterial agents is an essential alternative to chemicals (Santoyo *et al.*, 2012). However, a successful biofungicide must produce factors harmful to pathogens, and it is also necessary to deliver the beneficial bacteria to the right place at the right time. Therefore, endophytic bacteria have advantages over antagonists from other sources because they colonize host tissues internally without damaging their hosts or eliciting disease symptoms (Reinhold-Hurek and Hurek, 2011).

Although much research focuses on the exploitation of potentially bioactive bacterial endophytes, the endophytic bacteria of Chinese cabbage roots and their potential bioactivity

*For correspondence. E-mail: kmcho@gntech.ac.kr; Tel.: +82-55-751-3272; Fax: +82-55-751-3279
[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956

against phytopathogens has not been investigated. This study is the first to reveal the population structures of endophytic bacteria from the roots of Chinese cabbage cultivars in several areas of Seosang-gun, Haenam-gun, and in a transgenic plant from the laboratory. The extracellular enzymatic activity and *in vitro* antibiotic activity of the 653 endophytic isolates against food-borne pathogenic bacteria and phytopathogenic fungi were also investigated.

Materials and Methods

Microorganisms, plasmids, and media

The endophytic bacteria were isolated from Chinese cabbage roots (CCR) and cultured at 28°C or 37°C in tryptic soy (TS) medium and number 3 medium (No. 3: 10 g polypeptone, 10 g glucose, 1 g KH_2PO_4, and 0.5 g $MgSO_4{\cdot}7H_2O$ per 1 L, adjusted to pH 6.8) for antibiotic production. *Escherichia coli* DH5α and recombinant *E. coli* cells were cultured at 37°C in Luria-Bertani (LB) medium or LB medium supplemented with the appropriate antibiotics. The food-borne and plant pathogenic bacteria used were *E. coli* KCTC 1682, *Pseudomonas aeruginosa* KCTC 1750, *Salmonella enterica* KCTC 12456, *Salmonella enterica* ser. Enteritidis KCTC 12400, *Salmonella enterica* ser. Typhimurium KCTC 1925, *Shigella flexneri* KCTC 2008, *Shigella sonnei* KCTC 2518, *Bacillus cereus* KCTC 1012, *Listeria innocua* KCTC 3586, *Listeria ivanovii* KCTC 3444, *Listeria monocytogenes* KCTC 3569, and *Staphylococcus aureus* KCTC 1621. The pathogenic bacteria were collected from the Korean Collection of Type Cultures (KCTC). These bacteria were grown on TS (Tryptic soy) medium at 37°C. The phytophathogenic fungi *Rhizoctonia solani*, *Pythium ultimum*, *Phytophthora capsici*, and *Fusarium oxysporum* were kindly provided by the Laboratory of Phytopathology, Gyeongnam Agricultural Research and Extension Services, Jinju, Korea. The phytopathogenic fungi were maintained on potato dextrose agar (PDA) medium and were cultured at 28°C. The antibiotic ampicillin was purchased from Sigma and used at a concentration of 50 μg/ml. The LB, TS, and PDA media were purchased from Difco (Becton Dickinson Co). The pGEM-T Easy vector (Promega) was used for cloning and sequencing.

Isolation of Chinese cabbage root endophytic bacteria

Endophytic bacteria were isolated from Chinese cabbage roots (CCR). A total of ten plants of Chinese cabbage were randomly collected from each cultivars grown in Seosang-gun (SS, *Brassica campestris* L. ssp. p*ekinensis* cv. Geyodong), Haenam-gun (HN, *Brassica campestris* L. ssp. *pekinensis* cv. Dongpung) and from a transgenic plant (TP, *Brassica campestris* L. ssp. *pekinensis* cv. Kenshin) grown in a laboratory at the Gyeongnam Agricultural Research and Extension Services (GARES) center in the Jinju area of Korea. The surfaces of CCR were disinfected with 1% sodium hypochlorite for 10 min. The external portion of the roots (approximately 0.5 cm from the margin) was removed with a sterile blade, and the root tissue was triturated in a sterile porcelain mortar in sterile 10 mM phosphate buffer (pH 7.2). The root extracts were spread on TS agar and incubated at 28°C and 37°C for 48 h. The bacterial colonies were initially screened and grouped by colony color and morphological characteristics.

DNA isolation, amplification, sequencing, and recombinant DNA techniques

The isolated endophytic bacteria were cultured, then centrifuged at 14,000 × g_n at 4°C for 5 min. DNA was extracted from the pellet using the G-spinTM Genomic DNA Extraction Kit (iNtRON Biotechnology). The PCR primers used to amplify the 16S rRNA gene fragments were the universal primers (Forward, 5′-CGGAGAGTTTPATCCTPG-3′; reverse, 5′-TACGGCTACCTTPTTAGCGAC-3′). The 16S rRNA genes were amplified by PCR using the extracted DNA, Super-Therm DNA polymerase (JMR, Side Cup), 1.5 mM $MgCl_2$, 2 mM dNTP, and primers in a final volume of 50 μl over thirty cycles of denaturation at 94°C for 30 sec, annealing at 50°C for 30 sec, and extension at 72°C for 1 min 30 sec, followed by a final incubation at 72°C for 10 min. The anticipated PCR product was isolated and cloned, and its sequence was analyzed according to the method of and colleagues (Cho *et al*., 2007). Plasmid DNAs were isolated using a Plasmid DNA Purification Kit (iNtRON Biotechnology). Standard procedures for restriction endonuclease digestion, agarose gel electrophoresis, purification of DNA from agarose gels, DNA ligation, and other cloning-related techniques were used as previously described (Sambrook and Russel, 2001). Restriction enzymes and DNA-modifying enzymes were purchased from Gibco-BRL and Promega. All other chemicals were purchased from Sigma Chemical Co.

Cell-wall-degrading enzyme activity assay

The agar diffusion method was used for the detection of the extracellular cell-wall-degrading enzyme activity of the isolated CCR endophytic bacteria. The isolates were grown on different enzyme-activity indicator media for the detection of cellulase, xylanase, mannase, pectinase, amylase, protease, lipase, esterase, and DNase activity. The activities of these enzymes were estimated by measuring the diameter of the clear zone of the indicator medium, as previously described (Cho *et al*., 2007; Islam *et al*., 2010).

Antimicrobial activity assay

The agar disc diffusion technique was used for the determination of the antibacterial activity of the antibiotics produced by each isolate against the above-described food-borne bacteria (Barbosa *et al*., 2005). Paper disks were impregnated with 10 μl of bacterial suspension containing approximately 10^8 CFU/ml bacteria. The paper disks containing the bacterial suspension were placed on the plates, and the plates were incubated inverted at 28°C for 48 h. The antibacterial activity was estimated by measuring the diameter of the clear zone of growth inhibition.

An *in vitro* bioassay was conducted to evaluate the antagonistic properties of the endophytic bacteria isolated from CCR against the above-described phytopathogenic fungi by the paper disc method according to Cho and colleagues (Cho *et al*., 2007). Paper disks were impregnated with 10 μl of bacterial suspension containing approximately 10^8 CFU/ml bacteria. The paper disks containing the bacterial suspen-

Table 1. Similarity values of 16S rRNA gene sequences retrieved from the endophytic bacteria isolated from the interior root of Chinese cabbage

Isolates (Accession No.)	No. of isolates	Phylum	Nearest relatives[a] (Accession No.)	Similarity (%)
Seosang (118)				
SSR01 (EU373309)	2	Proteobacteria	*Xanthomonas* sp. BBCT38 (EF471219)	99.9
SSR02 (EU373311)	25	Proteobacteria	*Pseudomonas aeruginosa* PA7 (CP000744)	99.8
SSR03 (EU373312)	17	Proteobacteria	*Agrobacterium larrymoorei* (Z30542)	99.9
SSR04 (EU373313)	3	Proteobacteria	*Pseudomonas fluorescens* Pf29A (DQ473439)	99.9
SSR05 (EU373315)	13	LGCGPB[b]	*Bacillus clausii* KSM-K16 (AP006627)	99.8
SSR06 (EU373318)	6	LGCGPB	*Bacillus clausii* Y-76 (AB201796)	99.7
SSR07 (EU373329)	38	LGCGPB	*Bacillus pumilus* BPT-18 (EF523475)	99.6
SSR08 (EU373333)	6	HGCGPB[c]	*Corynebacterium* sp. 47081 (AF227825)	99.3
SSR09 (EU373335)	8	HGCGPB	*Microbacterium oxydans* P-2-63 (AB365061)	99.9
Haenam (302)				
HNR01 (EU373337)	1	Bacteriodetes	*Cytophaga* sp. MDA2507 (AY238333)	98.6
HNR02 (EU373338)	12	LGCGPB	*Bacillus* sp. CNJ815 PL04 (DQ448747)	99.9
HNR03 (EU373340)	9	LGCGPB	*Bacillus* sp. Bch1 (AF411118)	99.9
HNR04 (EU373342)	105	Proteobacteria	*Xanthomonas* sp. BBCT38 (EF471219)	99.9
HNR05 (EU373345)	4	HGCGPB	*Kocuria* sp. CNJ900 PL04 (DQ448710)	99.6
HNR06 (EU373346)	12	HGCGPB	*Corynebacterium* sp. 47081 (AF227825)	99.3
HNR07 (EU373351)	8	LGCGPB	*Bacillus* sp. KR2110 (AY822763)	99.6
HNR08 (EU373354)	61	HGCGPB	*Microbacterium hydrocarbonoxydans* BNP48 (AJ698726)	99.2
HNR09 (EU373356)	4	Proteobacteria	*Pseudomonas mediterranea* G-229-21T (EF673038)	98.8
HNR10 (EU373359)	2	LGCGPB	*Bacillus cereus* BGSC 6A5 (AY224388)	100.0
HNR11(EU373363)	8	LGCGPB	*Bacillus pumilus* BPT-18 (EF523475)	**93.9**[d]
HNR12 (EU373367)	5	Bacteriodetes	*Chryseobacterium* sp. YJ1 (DQ521273)	98.4
HNR13 (EU373369)	6	Proteobacteria	*Pseudomonas* sp. RRj228 (AY822762)	99.8
HNR14 (EU373372)	4	LGCGPB	*Staphylococcus* sp. H292 (AB177642)	99.9
HNR15 (EU373374)	5	Proteobacteria	*Pseudomonas* sp. An1 (AJ551142)	98.4
HNR16 (EU373377)	15	Proteobacteria	*Pseudomonas fluorescens* PfO-1 (CP000094)	99.6
HNR17 (EU373381)	1	LGCGPB	*Bacillus pumilus* CICCHLJ Q74 (EF528287)	99.9
HNR18 (EU373382)	17	Bacteriodetes	*Bacteriodetes bacterium* EC2 (AY337599)	**97.9**
HNR19 (EU373384)	1	LGCGPB	*Staphylococcus epidermidis* C4 (AM157427)	100.0
HNR20 (EU373387)	2	LGCGPB	*Bacillus amyloliquefaciens* BCRC 11266 (EF423605)	99.9
HNR21 (EU373388)	3	LGCGPB	*Bacillus pichinotyi* RS2 (AF519464)	99.8
HNR22 (EU373390)	8	Proteobacteria	*Pseudomonas synxantha* 2V5 (AM157452)	99.5
HNR23 (EU373392)	9	Proteobacteria	*Pseudomonas fluorescens* Pf29A (DQ473439)	99.9
Transgenic plant (233)				
TPR01 (EU373395)	30	Proteobacteria	*Shinella zoogloeoides* ATCC 19623 (X74915)	98.7
TPR02 (EU373397)	1	LGCGPB	*Bacillus subtilis* (AY887082)	99.7
TPR03 (EU373399)	2	LGCGPB	*Bacillus subtilis* MA139 (DQ415893)	99.9
TPR04 (EU373400)	27	HGCGPB	*Microbacterium oxydans* P-2-63 (AB365061)	99.8
TPR05 (EU373401)	9	LGCGPB	*Bacillus circulans* (AY043084)	99.1
TPR06 (EU373402)	5	LGCGPB	*Bacillus* sp. Bch1 (AF411118)	99.9
TPR07 (EU373403)	1	LGCGPB	*Bacillus pumilus* BPT-18 (EF523475)	99.5
TPR08 (EU373404)	12	LGCGPB	*Bacillus clausii* KSM-K16 (AP006627)	99.1
TPR09 (EU373406)	4	Proteobacteria	*Agrobacterium tumefaciens* JG 02 (DQ458962)	100.0
TPR10 (EU373410)	59	HGCGPB	*Microbacteriaceae bacterium* KVD-1982-06 (DQ490451)	99.9
TPR11 (EU373414)	8	LGCGPB	*Bacillus pumilus* SB 3182 (GU191909)	**93.1**
TPR12 (EU373419)	28	Proteobacteria	*Bosea* sp. BMA-4 (DQ855064)	100.0
TPR13 (EU373422)	2	HGCGPB	*Gordonia alkalivorans* (AB065369)	98.8
TPR14 (EU373424)	1	HGCGPB	*Micrococcus* sp. TUT1210 (AB188213)	99.9
TPR15 (EU373427)	3	Proteobacteria	*Acinetobacter* sp. EPas06 (EU252078)	99.4
TPR16 (EU373429)	25	LGCGPB	*Staphylococcus epidermidis* RP62A (CP000029)	99.7
TPR17 (EU373432)	8	Proteobacteria	Gram-negative bacterium DM 1 (AJ440749)	99.0
TPR18 (EU373436)	8	LGCGPB	*Bacillus pumilus* Y58 (EF203211)	99.9

[a] Ranges of 16S rRNA genes sequence is similarity values between endophytic bacteria and reference strain.
[b] LGCGPB: low G+C Gram-positive bacteria
[c] HGCGPB: high G+C Gram-positive bacteria
[d] Database sequences with > 98% similarity are shown in bold.

sion were placed on the plates, and the plates were incubated inverted at 28°C for 48 h. The antifungal activity was estimated by measuring the diameter of the clear zone of growth inhibition.

Results

Isolation and identification of CCR endophytic bacteria

The diversity of endophytic bacteria in the CC plants was evaluated in samples of roots from three different CC cultivars: two of these were grown in two different CC growing areas in Korea, namely Seosang (SS) and Haenam (HN), and the third is a transgenic plant (TP) grown in the laboratory. A total of 653 colonies were isolated from the interior roots of the CC samples collected from the three sampling sites (Table 1). Several genera of isolated bacteria were only present in one of the sampling sites. The isolated members of the genera *Xanthomonas*, *Pseudomonas*, *Corynebacterium*, *Agrobacterium*, and *Microbacterium* were all found only in the samples from SS. Members of the genera *Chryseobacterium*, *Cytophaga*, and *Kocuria* were found only in samples from HN, and the TP sample was the only one that contained members of the genera *Shinella*, *Micrococcus*, *Gordonia*, *Acinetobacter*, and *Bosea*. In particular, the 16S rRNA gene sequences of endophytes in three places, such as SS, HN, and TP roots, were from 73.5% to 99.9% similar to those found in homology matrix database.

The sample from the SS roots was estimated to contain 118 isolates representing 9 species. Among these SS root endophytes, *Bacillus pumilus* (SSR07) accounted for the highest number of bacterial isolates. In addition, *Pseudomonas aeruginosa* (SSR02) and *Agrobacterium larrymoorei* (SSR03) accounted for the majority of isolates from SS roots. In fact, the 16S rRNA gene sequences of SS root endophytes were each 99% similar to those found in databases.

Three hundred and two isolates representing 23 species were found from HN (Table 1). Among these endophytes, *Xanthomonus* sp. (HNR04) accounted for the highest number of isolates. Moreover, *Microbacterium hydrocarbonoxydans* (HNR08), an unidentified bacterium (HNR18) in the *Bacteriodetes*, and *Pseudomonas fluorescens* (HNR16) accounted for the majority of isolates from HN roots. Importantly, each of the 16S rRNA gene sequences of the 302 isolates was 93.9 to 100% similar to sequences in databases.

The endophytic bacterial samples isolated from TP comprised 233 isolates representing 18 species (Table 1), of which a bacterium (TPR10) in the *Microbacteriaceae* (TPR10) accounted for the highest number of isolates. In addition, *Shinella zoogloeoides* (TPR01), *Microbacterium oxydans* (TPR04), *Bosea* sp. (TPR12), and *Staphylococcus epidermidis* (TPR16) accounted for the majority of isolates from TP roots. These sequences were each 93.1 to 100% similar to those found in databases.

Phylogenetic analysis of CCR endophytic bacteria

Supplementary data Fig. S1A shows the phylogenetic analysis of the SS CCREB (Seosang Chinese cabbage root endophytic bacteria). In the 16S rRNA gene sequences of SS CCREB, three clusters can be described: HGC-GPB (high G+C Gram-positive bacteria), LGC-GPB (low G+C Gram-positive bacteria), and *Proteobacteria*. The first cluster, *i.e.*, the HGC-GPB cluster, was related to *Microbacterium* sp. (SSR09) and *Corynebacterium* sp. (SSR08). The second cluster, *i.e.*, LGC-GPB, was related to *Bacillus clausii* (SSR05, SSR06) and *Bacillus pumilus* (SSR07). Finally, the third cluster, namely the *Proteobacteria*, was related to *Agrobacterium larrymoorei* (SSR03), *Pseudomonas fluorescens* (SSR04), *Pseudomonas aeruginosa* (SSR02), and *Xanthomonas* sp. (SSR01).

Supplementary data Fig. S1B shows the phylogenetic analysis results of the HN CCREB (Haenam Chinese cabbage root endophytic bacteria). The 16S rRNA gene sequences of HN CCREB revealed four clusters: HGC-GPB, LGC-GPB, *Proteobacteria*, and *Bacteroidetes*. The first cluster, *i.e.*, the HGC-GPB was related to *Corynebacterium* sp. (HNR06), *Kocuria* sp. (HNR05), and *Microbacterium hydrocarbonoxydans* (HNR08). The second cluster, *i.e.*, LGC-GPB, was related to *Bacillus* sp. (HNR02, HNR07), *Bacillus amyloliquefaciens* (HNR20), *Bacillus pumilus* (HNR11, HNR17), *Bacillus pichinotyi* (HNR21), *Bacillus cereus* (HNR10), *Staphylococcus* sp. (HNR14), and *Staphylococcus epidermidis* (HNR19). The third cluster, *Proteobacteria*, was related to *Pseudomonas mediterranea* (HNR09), *Pseudomonas* sp. (HNR13, HNR15), *Pseudomonas fluorescens* (HNR16, HNR23), *Pseudomonas synxantha* (HNR22), and *Xanthomonas* sp. (HNR04), and the fourth cluster, namely Bacteroidetes was related to *Chryseobacterium* sp. (HNR12), an unidentified bacterium (HNR18) from the *Bacteriodetes*, and *Cytophaga* sp. (HNR01).

The results of phylogenetic analysis of the TP CCREB (Transgenic Plant Chinese cabbage root endophytic bacteria) are shown in Supplementary data Fig. S1C. The analysis of the 16S rRNA gene sequences of TP CCREB revealed three clusters. The HGC-GPB cluster was related to *Micrococcus* sp. (TPR12), *Microbacterium oxydans* (TPR04), *Microbacteriaceae bacterium* (TPR10), *Micrococcus* sp. (TPR14), and *Gordonia alkanivorans* (TPR13). The second cluster, *i.e.*, the LGC-GPB, was related to *Bacillus* sp. (TPR06), *B. subtilis* (TPR02, TPR03), *B. pumilus* (TPR07, TPR11, TPR18), *B. circulans* (TPR05), *B. clausii* (TPR08), and *S. epidermidis* (TPR16). The third cluster, *i.e.*, *Proteobacteria*, was related to *A. tumefaciens* (TPR09), *S. zoogloeoides* (TPR01), *Bosea* sp. (TPR12), and an unidentified Gram-negative bacterium (TPR17).

Extracellular hydrolytic enzyme activities of CCR endophytic bacteria

Isolates from the SS roots showed amylase, cellulase, xylanase, mannase, pectinase, DNase, protease, lipase, and esterase activities (Table 2). *B. pumilus* (SSR07) exhibited all extracellular enzyme activities tested except for pectinase. *Xanthomonas* sp. (SSR01) demonstrated all extracellular enzyme activities tested, with the exception of pectinase and lipase.

Isolates of the HN root samples showed amylase, cellulase, xylanase, mannase, pectinase, DNase, protease, lipase, and esterase activities (Table 2). *Bacillus* sp. (HNR03) had all of these extracellular enzyme activities. *B. pumilus* (HNR11) demonstrated all hydrolytic enzyme activities, except for pectinase. In addition, *B. pumilus* (HNR17) and *B. amyloliquefaciens* (HNR20) exhibited amylase, cellulase, xylanase, man-

Table 2. Identification of the various extracellular enzyme activity from the endophytic bacteria of the root of Chinese cabbage

Isolates	Nearest relatives	Enzyme activities[a]								
		Amylase	Cellulase	Xylanase	Mannase	Pectinase	DNase	Protease	Lipase	Esterase
Seosang (118)										
SSR01	*Xanthomonas* sp. BBCT38	+	+	+	+	-	+	++	-	+
SSR02	*P. aeruginosa* PA7	-	-	-	-	-	-	-	-	-
SSR03	*A. larrymoorei*	+	-	-	-	-	-	-	-	-
SSR04	*P. fluorescens* Pf29A	+	-	-	-	-	++	-	-	++
SSR05	*B. clausii* KSM-K16	-	-	-	-	-	-	-	-	-
SSR06	*B. clausii* Y-76	-	-	-	-	-	-	-	-	-
SSR07	*B. pumilus* BPT-18	+	++	+	+++	-	++	++	w	++
SSR08	*Corynebacterium* sp. 47081	+	-	-	-	+	+	-	-	-
SSR09	*M. oxydans* P-2-63	+	+	-	+	-	+	+	-	++
Haenam (302)										
HNR01	*Cytophaga* sp. MDA2507	+	w	-	-	-	+	++	-	-
HNR02	*Bacillus* sp. CNJ815 PL04	+	w	-	+	-	+	-	-	-
HNR03	*Bacillus* sp. Bch1	+++	+++	+	+	+	+	+++	w	+
HNR04	*Xanthomonas* sp. BBCT38	+	+	+	+	-	+	++	-	+
HNR05	*Kocuria* sp. CNJ900 PL04	-	-	-	-	-	++	-	-	-
HNR06	*Corynebacterium* sp. 47081	+	-	-	-	+	+	-	-	-
HNR07	*Bacillus* sp. KR2110	++	-	-	-	-	-	-	-	-
HNR08	*M. hydrocarbonoxydans* BNP48	++	-	-	++	-	++	++	-	-
HNR09	*P. mediterranea* G-229-21T	++	-	+	+	-	+	-	-	-
HNR10	*B. cereus* BGSC 6A5	+++	+	-	-	-	+	+++	-	-
HNR11	*B. pumilus* BPT-18	+	++	+	+++	-	+	+++	+	++
HNR12	*Chryseobacterium* sp. YJ1	++	-	-	-	-	-	+++	-	-
HNR13	*Pseudomonas* sp. RRj228	-	w	-	w	-	+	+	+	w
HNR14	*Staphylococcus* sp. H292	-	-	-	-	-	-	+	-	+++
HNR15	*Pseudomonas* sp. An1	-	-	-	+++	-	-	-	-	-
HNR16	*P. fluorescens* PfO-1	-	-	-	-	-	+	+	+	++
HNR17	*B. pumilus* CICCHLJ Q74	w	++	+++	+	-	++	++	-	+++
HNR18	*B. bacterium* EC2	-	w	-	-	-	-	+++	-	++
HNR19	*S. epidermidis* C4	+	-	-	-	-	w	+++	-	++
HNR20	*B. amyloliquefaciens* BCRC 11266	+++	+	+	+	-	++	+++	-	++
HNR21	*B. pichinotyi* RS2	-	-	-	-	-	++	-	-	-
HNR22	*P. synxantha* 2V5	-	-	-	-	+	+++	+	-	-
HNR23	*P. fluorescens* Pf29A	-	w	-	-	-	-	-	++	-
Transgenic plant (223)										
TPR01	*S. zoogloeoides* ATCC 19623	-	+	-	+	-	-	-	-	w
TPR02	*B. subtilis*	++	++	-	-	+	+	++	+	+
TPR03	*B. subtilis* MA139	++	++	+	+	+	+	++	+	+
TPR04	*M. oxydans* P-2-63	+	+	-	+	-	+	+	-	++
TPR05	*B. circulans*	-	+	+	-	-	-	+	-	-
TPR06	*Bacillus* sp. Bch1	++	++	+	+	+	+	++	+	+
TPR07	*B. pumilus* BPT-18	+	+	++	+	+++	+	+	-	+
TPR08	*B. clausii* KSM-K16	-	-	-	-	-	-	-	-	-
TPR09	*A. tumefaciens* JG 02	w	+	+	w	-	-	-	+	+
TPR10	*M. bacterium* KVD-1982-06	-	-	-	-	w	w	-	-	-
TPR11	*B. pumilus* SB 3182	+	+	+	+	-	+	+	-	+
TPR12	*Bosea* sp. BMA-4	+	-	++	-	-	-	-	-	-
TPR13	*Gordonia alkalivorans*	-	-	++	-	-	-	-	-	-
TPR14	*Micrococcus* sp. TUT1210	-	w	-	-	-	-	+++	-	-
TPR15	*Acinetobacter* sp. EPas06	-	-	+	-	-	-	-	+	+
TPR16	*S. epidermidis* RP62A	+	-	-	-	-	-	+++	-	++
TPR17	Gram-negative bacterium DM 1	-	-	-	+	+	-	-	-	-
TPR18	*B. pumilus* Y58	++	+	+	+	+	+	+	+	++

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

Table 3. ***In vitro*** **inhibitory activity[a] against the food-borne pathogenic bacteria by Chinese cabbage root endophytic bacteria (CCREB)**

Isolates	Nearest relatives	Human food-borne pathogenic bacteria[b]											
		Eci	Sea	Ses	Stm	Sfi	Ssi	Paa	Bcs	Lia	Lii	Lms	Sas
Seosang (118)													
SSR01	*Xanthomonas* sp. BBCT38	-	-	-	-	-	-	-	-	-	-	-	-
SSR02	*P. aeruginosa* PA7	-	-	-	-	-	-	-	-	-	-	-	-
SSR03	*A. larrymoorei*	-	-	-	-	-	-	-	-	-	-	-	-
SSR04	*P. fluorescens* Pf29A	-	-	-	-	-	-	-	9.6	-	-	-	-
SSR05	*B. clausii* KSM-K16	-	-	10.2	-	-	-	11.4	-	-	-	-	-
SSR06	*B. clausii* Y-76	-	-	-	-	-	-	-	-	-	-	-	-
SSR07	*B. pumilus* BPT-18	-	11.2	10.8	13.2	15.6	13.0	-	16.0	12.1	17.0	12.2	15.2
SSR08	*Corynebacterium* sp. 47081	-	-	-	-	-	-	-	-	-	-	-	-
SSR09	*M. oxydans* P-2-63	-	-	-	-	-	-	-	-	8.8	-	-	-
Haenam (302)													
HNR01	*Cytophaga* sp. MDA2507	-	-	-	-	-	-	-	-	-	-	-	-
HNR02	*Bacillus* sp. CNJ815 PL04	-	-	-	-	-	-	-	-	-	+	-	-
HNR03	*Bacillus* sp. Bch1	8.4	11.2	10.4	13.2	16.8	12.2	-	11.4	-	10.6	12.0	14.8
HNR04	*Xanthomonas* sp. BBCT38	-	-	-	-	-	-	-	-	-	-	-	-
HNR05	*Kocuria* sp. CNJ900 PL04	-	-	-	-	-	-	-	9.6	-	10.2	-	-
HNR06	*Corynebacterium* sp. 47081	-	-	-	-	-	-	-	-	-	-	-	-
HNR07	*Bacillus* sp. KR2110	-	-	-	-	-	-	-	11.8	-	-	-	-
HNR08	*M. hydrocarbonoxydans* BNP48	-	-	-	-	-	-	-	-	-	-	-	-
HNR09	*P. mediterranea* G-229-21T	-	-	-	-	-	12.2	-	-	-	-	-	11.8
HNR10	*B. cereus* BGSC 6A5	-	-	-	-	-	-	-	-	12.0	-	10.2	11.0
HNR11	*B. pumilus* BPT-18	-	12.4	11.6	12.2	13.2	9.6	-	14.8	15.2	-	10.0	9.8
HNR12	*Chryseobacterium* sp. YJ1	-	-	-	-	-	-	-	-	-	-	-	-
HNR13	*Pseudomonas* sp. RRj228	-	-	-	-	-	-	-	17.0	15.4	-	11.0	10.2
HNR14	*Staphylococcus* sp. H292	-	-	-	-	-	-	-	-	8.8	-	-	10.2
HNR15	*Pseudomonas* sp. An1	-	-	-	-	-	-	-	9.8	-	-	11.0	10.4
HNR16	*P. fluorescens* PfO-1	-	-	-	-	-	-	-	10.6	-	-	10.8	12.0
HNR17	*B. pumilus* CICCHLJ Q74	-	-	-	-	-	8.8	17.8	-	-	-	-	14.6
HNR18	*B. bacterium* EC2	-	-	-	-	-	-	-	11.2	-	-	-	10.6
HNR19	*S. epidermidis* C4	-	-	-	-	-	-	-	-	-	-	-	-
HNR20	*B. amyloliquefaciens* BCRC 11266	-	-	-	-	-	-	-	-	-	-	-	-
HNR21	*B. pichinotyi* RS2	-	-	-	-	-	-	-	-	-	-	-	-
HNR22	*P. synxantha* 2V5	-	-	-	-	-	-	-	-	-	-	-	-
HNR23	*P. fluorescens* Pf29A	-	-	-	-	-	-	-	10.0	-	-	-	-
Transgenic plant (223)													
TPR01	*S. zoogloeoides* ATCC 19623	-	-	-	-	-	-	-	-	-	-	-	-
TPR02	*B. subtilis*	8.4	-	-	12.4	-	-	-	-	-	-	13.2	11.6
TPR03	*B. subtilis* MA139	8.6	12.0	11.8	14.0	15.2	11.6	-	16.8	10.0	11.4	12.2	15.0
TPR04	*M. oxydans* P-2-63	-	-	-	-	-	-	-	-	9.8	-	-	-
TPR05	*B. circulans*	-	-	-	-	-	-	-	-	-	-	-	-
TPR06	*Bacillus* sp. Bch1	8.2	11.4	12.0	11.6	10.6	10.2	-	14.2	-	11.0	15.2	11.2
TPR07	*B. pumilus* BPT-18	-	10.0	11.0	11.8	12.1	9.8	-	15.4	11.8	12.0	11.6	13.2
TPR08	*B. clausii* KSM-K16	-	-	10.4	-	-	-	9.6	-	-	-	-	-
TPR09	*A. tumefaciens* JG 02	-	-	-	-	-	-	-	-	-	-	-	-
TPR10	*M. bacterium* KVD-1982-06	-	-	-	-	-	-	-	-	-	-	-	-
TPR11	*B. pumilus* SB 3182	-	-	-	9.2	-	-	10.6	10.4	-	-	11.2	-
TPR12	*Bosea* sp. BMA-4	-	-	-	-	-	-	-	11.2	-	-	-	-
TPR13	*Gordonia alkalivorans*	-	-	-	-	-	-	-	-	-	-	-	-
TPR14	*Micrococcus* sp. TUT1210	-	-	-	-	-	-	-	-	-	-	-	-
TPR15	*Acinetobacter* sp. EPas06	-	-	-	-	-	-	-	-	-	-	-	-
TPR16	*S. epidermidis* RP62A	-	-	-	-	-	-	-	-	-	-	-	-
TPR17	Gram-negative bacterium DM 1	-	-	-	-	-	-	-	-	-	-	-	-
TPR18	*B. pumilus* Y58	-	-	-	-	-	-	-	10.0	-	-	11.4	-

[a] The antibacterial activity was estimated by measuring the diameter of the clear zone (including paper disc, 8 mm diameter) of growth inhibition.
[b] Food-borne pathogenic bacteria: Eci, *Eschericia coli* KCTC 1682; Paa, *Pseudomonas aeruginosa* KCTC 1750; Sea, *Salmonella enterica* KCTC 12456; Ses, *Salmonella enteritids* KCTC 12400; Stm, *Salmonella typhimerium* KCTC 1925; Sfi, *Shigella flexineri* KCTC 2008; Ssi, *Shigella sonnei* KCTC 2518; Bcs, *Bacillus cereus* KCTC1012; Lia, *Listeria innocula* KCTC 3586; Lii, *Listeria ivanovii* KCTC 3444; Lms, *Listeria monocytogenes* KCTC 3569; Sas, *Staphylococcus aureus* KCTC 1621.

Table 4. ***In vitro*** **inhibitory activity[a] against the plant pathogenic fungi by Chinese cabbage root endophytic bacteria (CCREB)**

Isolates	Nearest relatives	Plant pathogenic fungi[b]			
		Pca	Fox	Rso	Pul
Seosang (118)					
SSR01	*Xanthomonas* sp. BBCT38	-	-	-	-
SSR02	*P. aeruginosa* PA7	-	-	-	-
SSR03	*A. larrymoorei*	-	-	-	-
SSR04	*P. fluorescens* Pf29A	-	12.1	-	13.8
SSR05	*B. clausii* KSM-K16	-	-	-	9.2
SSR06	*B. clausii* Y-76	-	-	-	9.4
SSR07	*B. pumilus* BPT-18	-	9.8	14.2	9.6
SSR08	*Corynebacterium* sp. 47081	-	-	-	-
SSR09	*M. oxydans* P-2-63	8.4	8.9	8.8	-
Haenam (302)					
HNR01	*Cytophaga* sp. MDA2507	-	-	-	8.4
HNR02	*Bacillus* sp. CNJ815 PL04	-	-	-	-
HNR03	*Bacillus* sp. Bch1	12.4	14.6	9.6	8.6
HNR04	*Xanthomonas* sp. BBCT38	-	-	-	-
HNR05	*Kocuria* sp. CNJ900 PL04	-	-	-	-
HNR06	*Corynebacterium* sp. 47081	-	-	-	-
HNR07	*Bacillus* sp. KR2110	-	-	9.7	-
HNR08	*M. hydrocarbonoxydans* BNP48	-	-	-	-
HNR09	*P. mediterranea* G-229-21T	-	-	-	-
HNR10	*B. cereus* BGSC 6A5	-	13.2	14.1	10.6
HNR11	*B. pumilus* BPT-18	-	10.2	14.65	9.0
HNR12	*Chryseobacterium* sp. YJ1	-	8.3	-	-
HNR13	*Pseudomonas* sp. RRj228	-	14.8	9.8	12.6
HNR14	*Staphylococcus* sp. H292	-	-	-	-
HNR15	*Pseudomonas* sp. An1	-	-	-	-
HNR16	*P. fluorescens* PfO-1	-	11.8	-	15.4
HNR17	*B. pumilus* CICCHLJ Q74	-	10.2	11.4	9.6
HNR18	*B. bacterium* EC2	-	-	-	-
HNR19	*S. epidermidis* C4	-	-	-	-
HNR20	*B. amyloliquefaciens* BCRC 11266	-	-	-	-
HNR21	*B. pichinotyi* RS2	-	-	-	-
HNR22	*P. synxantha* 2V5	-	-	-	-
HNR23	*P. fluorescens* Pf29A	-	10.8	-	14.2
Transgenic plant (223)					
TPR01	*S. zoogloeoides* ATCC 19623	-	-	-	-
TPR02	*B. subtilis*	11.2	12.6	16.2	12.2
TPR03	*B. subtilis* MA139	12.1	12.4	15.8	14.6
TPR04	*M. oxydans* P-2-63	8.2	9.2	9.0	-
TPR05	*B. circulans*	-	8.8	9.4	-
TPR06	*Bacillus* sp. Bch1	12.0	14.1	10.6	8.4
TPR07	*B. pumilus* BPT-18	-	+	+	+
TPR08	*B. clausii* KSM-K16	-	-	10.4	-
TPR09	*A. tumefaciens* JG 02	-	-	-	-
TPR10	*M. bacterium* KVD-1982-06	-	-	-	-
TPR11	*B. pumilus* SB 3182	-	13.2	9.2	-
TPR12	*Bosea* sp. BMA-4	-	-	-	-
TPR13	*Gordonia alkalivorans*	-	-	-	-
TPR14	*Micrococcus* sp. TUT1210	-	-	-	-
TPR15	*Acinetobacter* sp. EPas06	-	-	-	-
TPR16	*S. epidermidis* RP62A	-	-	-	-
TPR17	Gram-negative bacterium DM 1	-	-	-	-
TPR18	*B. pumilus* Y58	-	9.4	10.2	-

[a] The antifungal activity was estimated by measuring the diameter of the clear zone (including paper disks, 8 mm diameter) of growth inhibition.
[b] Plant pathogenic fungi: Pca, *Phytophthora capsici*; Fox, *Fusarium oxysporum*; Rso, *Rhizoctonia solani*; Pul, *Phytophthora ultimum*.

nase, DNase, protease, and esterase activities. *Bacillus* sp. (HNR03), *B. cereus* (HNR10), *B. pumilus* (HNR11, HNR17), *Chryseobacterium* sp. (HNR12), *B. bacterium* (HNR18), *S. epidermidis* (HNR19) and *B. amyloliquefaciens* (HNR20) showed moderate protease activity.

Isolates of TP root samples showed amylase, cellulase, xylanase, mannase, pectinase, DNase, protease, lipase, and esterase activities (Table 2). *B. subtilis* (TPR03), *Bacillus* sp. (TPR06), and *B. pumilus* (TPR18) exhibited all of the extracellular enzyme activities. *B. pumilus* (TPR07) exhibited all hydrolytic enzyme activities with the exception of lipase. The HN root samples presented the highest level of extracellular hydrolytic enzyme activity (Supplementary data Fig. S2).

Antagonism of CCR endophytic bacteria to food-borne pathogenic bacteria

The antibacterial activity of the isolated Chinese cabbage root endophytic bacteria (CCREB) was evaluated against food-borne pathogens, such as *E. coli*, *P. aeruginosa*, *S. enterica*, *S.* Enteritidis, *S.* Typhimurium, *S. flexneri*, *S. sonnei*, *B. cereus*, *L. innocua*, *L. ivanovii*, *L. monocytogenes*, and *S. aureus* (Table 3). Among the bacterial isolates from the root samples, *Bacillus* sp. (HNR03, TPR06), *B. pumilus* (SSR07, HNR11, TPR-07), and *B. subtilis* (TPR03) showed high antibacterial activity against most of the food-borne pathogens tested. In addition, *Bacillus* sp. (HNR03, TPR06), *B. subtilis* (TPR02, TPR03) demonstrated antibacterial activity against *E. coli* (Table 3). The HN root samples exhibited the highest populations of bacteria with antibacterial activity against food-borne pathogens (Supplementary data Fig. S3).

Antagonism of CCR endophytic bacteria to fungal phytopathogens

The three CCEBs (SS CCREB, HN CCREB, and TP CCREB) were studied to determine their *in vitro* inhibitory activity against *Rhizoctonia solani*, *Fusarium oxysporum*, *Pythium ultimum,* and *Phytophthora capsici* (Table 4). Two species, namely *Bacillus* sp. (HNR03, TPR06), *B. subtilis* (TPR02, TPR-03) appeared to have a broad spectrum of antifungal activity, as determined through *in vitro* tests. These bacteria exhibited a strong antifungal effect on all phytopathogens tested, including *P. ultimum*, *P. capsici, F. oxysporum* and *R. solani*. In addition, *B. cereus* (HNR10), and *B. pumilus* (SSR07, HNR11, HNR17, TPR07) demonstrated antifungal activity against *P. ultimum*, *F. oxysporum*, and *R. solani* with the exception of *P. capsici*. Moreover, *M. oxydans* (SSR09) presented antifungal activity against *P. capsici*, and *F. oxysporum*, and *R. solani,* but not against *P. ultimum* (Table 4). The SS root samples presented the highest population of bacteria with antifungal activity against phytopathogens (Supplementary data Fig. S4).

Discussion

Several studies of bacterial endophytes have been focused on agriculturally and medicinally important plant roots (Cho *et al.*, 2007; Islam *et al.*, 2010; Lkeda *et al.*, 2013), while the literature on bacterial endophytes from Chinese cabbage roots is sparse. The results of the present study describe the composition of the endophyte communities recovered from Chinese cabbage (*Brassica campestris*) roots from cultivars grown in Seosang-gun and Haenam-gun and from a transgenic plant grown in a laboratory at GARES in the Jinju area of Korea. In this study, 653 different endophytic isolates belonging to 19 different bacterial genera were identified. *Xanthomonas* sp. BBCT38, *Bacillus pumilus* BPT-18, and *Corynebacterium sp.* 47081 were obtained from both the SS and the HN root samples. However, more isolates of *Xanthomonas* sp. BBCT38 and *Corynebacterium sp.* 47081 were obtained from the SS root sample than from the HN root sample. Similarly, *Bacillus clausii* KSM-K16 and *Microbacterium oxydans* were obtained in both the SS and TP root samples, but more of those isolates were obtained from the TP root sample than from the SS root sample. *Bacillus* sp. Bch1 was isolated from both the HN and TP root samples, and more isolates of *Bacillus* sp. Bch1 were obtained from HN than from TP. Notably, *Bacillus pumilus* BPT-18 related bacteria were commonly found in samples from all three locations, but the number of its isolates was much larger in the SS sample than in either the TP or SS samples. The total number of isolates obtained from HN root samples was larger than the number obtained from SS or TP root samples, indicating that the roots of Chinese cabbage grown in the Haenam-gun area were the most suitable niche for the bacterial endophytes. Several studies have addressed changes in the composition and abundance of bacterial populations, especially lactic acid bacteria (LAB), during the different stages of Chinese cabbage *kimchi* fermentation (Kim and Chun, 2005; Jung *et al.*, 2012; Hong *et al.*, 2013). In addition to LABs, *Streptococus salivarius*, *Bacillus subtilis* and some uncultured bacteria were identified in Chinese cabbage *kimchi* fermentation (Hong *et al.*, 2013). Importantly, none of these LABs except *Bacillus* spp. appeared in the endophytic bacterial community of SS, HN, and TP root samples. However, several isolates from SS, HN, and TP Chinese cabbage roots have shown high similarities with *Bacillus* sp. Some other isolates from SS and HN Chinese cabbages have shown high similarities with *Bacillus subtilis*. It was reported that *Bacillus* spp. were identified in the initial stage of *kimchi* fermentation (An *et al.*, 1999; Jung *et al.*, 2014). Very recently, Takahashi and colleagues reported that bacteria belonging to the genera *Achromobacter*, *Arthrobacter*, *Corynebacterium*, and *Curtobacterium* were detected from all three types of beer (all-malt, half- and low-malt) at mashing or before boiling, while *Acinetobacter and Bacillus* were detected from all samples during fermentation (Takahashi *et al.*, 2015). The present study suggests that isolates from SS, HN, and TP roots related to *Bacillus* spp., *Acinetobacter,* and *Corynebacterium* are related to bacteria that are involved in *kimchi* fermentation.

The 16S rRNA gene sequencing revealed the presence of endophytic bacteria in the Chinese cabbage roots belonging to the HGCGPB, LGCGPB, *Proteobacteria*, and *Bacteroidetes*. Among those, the genera *Bacillus* and *Pseudomonas* were dominant. The bacteria belonging to some of the identified genera, such as *Pseudomonas* and *Bacillus*, are easy to culture, and may provide plants with protection against pathogen attacks through various modes of action, and cultivation-dependent studies have identified them as frequently occur-

ring endophytes (Seghers *et al*., 2004; Cho *et al*., 2007). These features have led to the increasingly common design and implementation of antimicrobial biological products based on *Bacillus* species or their metabolites as alternative to synthetic chemicals for plant disease control (Schisler *et al*., 2004).

The endophytic bacteria isolated from the roots of the three cultivars of Chinese cabbage exhibited various extracellular enzyme activities. Specifically, the endophytic bacteria in the roots collected from the Haenam cultivation area presented high extracellular enzymatic activity (supplementary data Fig. S2). A wide range of cell-wall-degrading enzymes were produced by isolates from the three cultivars of the genus *Bacillus*. In general, the hydrolytic enzymes of endophytes are thought to be important for their colonization of plant roots (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). In a related study, Verma and colleagues showed the presence of different levels of cellulase and pectinase activities in different isolates, suggesting their potential for inter/intracellular colonization (Verma *et al*., 2001). The constitutive release of plant-cell-wall-degrading enzymes by endophytic bacteria is undesirable because this would likely lead to pathogenicity. Therefore, it is hypothesized that hydrolytic enzymes are only produced by endophytes during the early invasion phase and that their production is discontinued after the endophytes take up residence in plant tissue.

In this study, strong *in vitro* inhibition of the tested food-borne pathogenic bacteria was obtained with *Bacillus* sp. (HNR03, TPR06), *B. pumilus* (SSR07, HNR11, TPR07), and *B. subtilis* (TPR03). Importantly, all of these *Bacillus* genera had shown cell-wall-degrading enzyme activities. The antimicrobial peptide of *Bacillus subtilis* isolated from Chinese fermented foods has been found to inhibit the food-borne pathogen *Vibrio parahaemolyticus* (Pu *et al*., 2013). Therefore, it can be hypothesized that Chinese cabbage roots are a suitable niche for bacterial endophytes displaying antagonistic activity against food-borne pathogenic bacteria.

Cell-wall-degrading enzymes, such as β-1,3-glucanase, cellulase, proteases and chitinases, are involved in the antagonistic activity of some biological control agents against phytopathogenic fungi (Cherif *et al*., 1993). In fact, *Bacillus pumilus* (SSR07, HNR11, HNR17, TPR11), *Microbacterium oxydans* (SSR09, TPR04), *Bacillus* sp. (HNR03, TPR06), *Bacillus cereus* (HNR10), *Bacillus subtilis* (TPR02, TPR03), and *Pseudomonas* (HNR13) showed strong antagonistic activity *in vitro* towards the pathogenic fungi tested. In contrast, *Bacillus clausii* (SSR05, SSR06) showed no extracellular enzyme activity, and displayed antagonism only towards *P. ultimum*. In a related study, *Bacillus subtilis* K1, *B. amyloliqufaciens* A13, and *Bacillus* sp. A32 displayed antifungal activity by secreting iturin, fencycins and surfactins (Pathak and Keharia, 2013). Therefore, it is hypothesized that the suppression of *P. ultimum* growth (*in vitro*) by *Bacillus clausii* (SSR05, SSR06) occurred through an alternate mechanism, such as the action of cyclic lipopeptides (e.g., ituirin, fengycins) or of volatile compounds, or via oxidative defense. Among the tested phytopathogenic fungi, *F. oxysporum* is most likely to invade the Chinese cabbage (Khastini *et al*., 2012). Notably, the endophytes *Pseudomonas fluorescens* (SSR04, HNR16), *Bacillus* sp. (HNR03), *Bacillus cereus* (HNR10), *Pseudomonas* sp. (HNR13), *Bacillus subtilis* (TPR02, TPR03), *Bacillus* sp. (TPR06), and *Bacillus pumilus* (TPR11) displayed remarkably antagonistic activity *in vitro* towards *F. oxysporum*. In addition, *F. oxysporum* antagonists were more abundant in transgenic plants and Haenam roots than in Seosang roots of Chinese cabbage, which suggested that roots of the transgenic plant and of the Haenam cultivar are favor the growth and development of the endophytes and invites them to inhibit the attack of the phytopathogenic fungi.

The potential use of endophytic bacteria for the biocontrol of fungal or bacterial diseases has only been investigated in a limited number of host crops, but is of special interest because the same bacterium may both promote the growth of the host and provide biological control of pathogens. In particular, a variety of soil microorganisms, including bacteria and fungi, have demonstrated potential as biocontrol agents against *Fusarium oxysporium* (Pereira *et al*., 2007; Moretti *et al*., 2008; Khastini *et al*., 2012). Meanwhile, Khastini and colleagues have reported the suppression of *Fusarium* wilt in Chinese cabbage using the endophytic fungus *Veronaeopsis simplex* Y34 (Khastini *et al*. 2012). However, there is some evidence that endophytic bacteria, such as *Pseudomonas* and *Bacillus*, can present protective antifungal properties as well (Berg *et al*., 2005; Cho *et al*., 2007; Seo *et al*., 2010). Among endophytic bacteria, *Bacillus* sp. is stable in soil as spores, and this is advantageous for their use as biocontrol agents because of the spores' stability; other advantages include their ease of handling and their production of lipopeptides that specifically suppress fungal spores. Cho and colleagues suggested that three isolated endophytic bacteria from ginseng, namely *P. polymyxa* (GS01), *Bacillus* sp. (GS07), and *Pseudomonas poae* (JA01), show potential activity as biocontrol agents against phytopathogenic fungi (Cho *et al*., 2007). Therefore, the *Fusarium oxysporum* antagonists (genera *Bacillus*, *Pseudomonas*) identified in this study are hypothesized to be potential biocontrol agents.

In the case of antifungal endophytes, the host plant benefits from association with endophytes because the endophytes' antifungal activity provides some protection against fungal infection. The complex *Rhizobium*-legume symbiosis is known to involve a series of molecules produced by the host plant that lead to the exchange of recognition signals. However, it has only recently been recognized that endophytic bacteria play an important role in resistance to disease and that signals exist to mediate communication between the endophyte and its host (Cho *et al*., 2007). Furthermore, Lacava and colleagues reported that the growth of the phytopathogenic bacteria *Xylella fastidiosa* is stimulated by endophytic *Methylobacterium extorquens* and inhibited by endophytic *Curtobacterium flaccumfaciens* (Lacava *et al*., 2004). One important factor that has been postulated for the optimal performance of an introduced endophytic microbial is the relationship between the plant genotype and the effective colonization of the host (Sturz and Nowak, 2000).

This study determined that there are regional differences in the root interior microbial community of Chinese cabbage. Most of the isolates belonging to the genus *Bacillus* presented promising activity against food-borne pathogenic bacteria

and phytopathogenic fungi. In particular, *Bacillus* sp. (HNR-03, TPR06), *B. subtilis* (TPR02, TPR03), *Bacillus cereus* (HNR-10), *Pseudomonas fluorescens* (SSR04), and *Pseudomonas* sp. (HNR13) showed promising antagonism towards mycelial growth *in vitro* of the tested fungal pathogens *P. capsici*, *F. oxysporum*, *R. solani*, and *P. ultimum*. These biocontrol profiles suggested that the above-mentioned endophytes singly or in combination protect the Chinese cabbage roots from the attacks of the tested phytopathogens in different stages of its life-cycle. Further studies are needed to understand the possible development of symptoms that may occur after the re-inoculation of the endophytes under conducive (e.g., greenhouse and field) conditions to improve our understanding of the complex plant-microbe interactions in this system.

Acknowledgements

This work was supported by Gyeongnam National University of Science and Technology Grant (2015 year), Republic of Korea.

References

An, D.J., Lew, K., and Lee, K.P. 1999. Effects of adipic acid and storage temperature on extending the shelf life of kimchi. *Food Sci. Biotechnol.* **8**, 78–82.

Al-Mallah, M.K., Davey, M.R., and Cooking, E.C. 1987. Enzymatic treatment of clover root hairs removes a barrier to *Rhizobium*-host specificity. *Nat. Biotechnol.* **5**, 1319–1322.

Andrade, L.A., de Souza, G.L.O.D., Nietsche, S., Xavier, A.A., Costa, M.R., Cardosa, A.S.M., Pereira, M.C.T., and Pereira, D.F.G.S. 2014. Analysis of the abilities of endophytic bacteria associated with banana tree roots to promote plant growth. *J. Microbiol.* **52**, 27–34.

Barbosa, T.M., Serra, C.R., Ragione, R.M.L., Woodward, M.J., and Henriques, A.O. 2005. Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Appl. Environ. Microbiol.* **71**, 968–978.

Berg, G., Krechel, A., Ditz, M., Sikora, R.A., 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.

Cherif, M., Benhamou, N., and Belanger, R.R. 1993. Occurrence of cellulose and chitin in the hyphal walls of *Pythium ultimum*: a comparative study with other plant pathogenic fungi. *Can. J. Microbiol.* **39**, 213–222.

Cho, K.M., Hong, S.Y., Lee, S.M., Kim, Y.H., Kahng, G.G., Lim, Y.P., Kim, H., and Yun, H.D. 2007. Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. *Microbial. Ecol.* **54**, 341–351.

Dalton, D.A., Kramer, S., Azios, N., Fusaro, S., Cahill, E., and Kennedy, C. 2004. Endophytic nitrogen fixation in dune grasses (*Ammophila arenaria* and *Elymus mollis*) from Oregon. *FEMS Microbiol. Ecol.* **49**, 469–479.

de Melo Pereira, G.V., Magalhaes, K.T., Lorenzetii, E.R., Souza, T.P., and Schwan, R.F. 2012. A multiphasic approach for the identification of endophytic bacterial in strawberry fruit and their potential for plant growth promotion. *Microb. Ecol.* **63**, 405–417.

Falcao, L.L., Silva-Werneck, J.O., Vilarinho, B.R., da Silva, J.P., Pomella, A.W.V., and Marcellino, L.H. 2014. Antimicrobial and plant growth-promoting properties of the cacao endophyte *Bacillus subtilis* ALB629. *J. Appl. Microbiol.* **116**, 1584–1592.

Fukui, Y., Abe, M., Kobayashi, M., Yano, Y., and Satomi, M. 2014. Isolation of *Hyphomonas* strains that induce normal morphogenesis in protoplasts of the marine red algae *Pyropia yezoensis*. *Microb. Ecol.* **68**, 556–566.

Haque, M.A., Lee, J.H., and Cho, K.M. 2015. Endophytic bacterial diversity in Korean kimchi made of Chinese cabbage leaves and their antimicrobial activity against pathogens. *Food Cont.* **56**, 24–33.

Hong, Y., Yang, H.S., Chang, H.C., and Kim, H.Y. 2013. Comparison of bacterial community changes in fermenting kimchi at two different temperatures using denaturing gradient gel electrophoresis analysis. *J. Microbiol. Biotechnol.* **23**, 76–84.

Islam, S.M.A., Math, R.K., Kim, J.M., Yun, M.G., Cho, J.J., Kim, E.J., Lee, Y.H., and Yun, H.D. 2010. Effect of plant age on endophytic bacterial diversity of balloon flower (*Platycodon grandiflorum*) root and their antimicrobial activities. *Curr. Microbiol.* **61**, 346–356.

Jung, J.Y., Lee, S.H., and Jeon, C.O. 2014. Kimchi microflora: history, current status, and perspectives for industrial kimchi production. *Appl. Microbiol. Biotechnol.* **98**, 2385–2393.

Jung, J.Y., Lee, S.H., Lee, H.J., Seo, H.Y., Park, W.S., and Jeon, C.O. 2012. Effects of *Leuconostoc mesenteroides* starter cultures on microbial communities and metabolities during kimchi fermentation. *Int. J. Food Microbiol.* **153**, 378–387.

Khan, A.L., Waqas, M., Kang, S.M., Al-Harrasi, A., Hussain, J., Al-Rawahi, A., Al-Khiziri, S., Ullah, I., Ali, L., Jung, H.Y., *et al.* 2014. Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *J. Microbiol.* **52**, 689–695.

Khastini, R.O., Ohta, H., and Narisawa, K. 2012. The role of a dark septate endophytic fungus, *Veronaeopsis simplex* Y34, in Fusarium disease suppression in Chinese cabbage. *J. Microbiol.* **50**, 618–624.

Kim, M. and Chun, J. 2005. Bacterial community structure in kimchi, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *Int. J. Food Microbiol.* **103**, 91–96.

Lacava, P.T., Araujo, W.L., Marcon, J., Maccheroni, W. Jr., and Azevedo, J.L. 2004. Interaction between endophytic bacteria from citrus plants and the phytopathogenic bacteria *Xylella fastidiosa*, causal agent of citrus-variegated chlorosis. *Lett. Appl. Microbiol.* **39**, 55–59.

Lkeda, A.C., Bassani, L.L., Adamoski, D., Stringari, D., Cordeiro, V.K., Glienke, C., Steffens, M.B.R., Hungria, M., and Galli-Terasawa, L.V. 2013. Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. *Microb. Ecol.* **65**, 154–160.

Li, C.H., Shi, L., Han, Q., Hu, H.L., Zhao, M.W., Tang, C.M., and Li, S.P. 2012. Biocontrol of verticillium wilt and colonization of cotton plants by an endophytic bacterial isolate. *J. Appl. Microbiol.* **113**, 641–651.

Li, C.H., Zhao, M.W., Tang, C.M., and Li, S.P. 2010. Population dynamics and identification of endophytic bacteria antagonistic toward plant-pathogenic fungi in cotton root. *Microb. Ecol.* **59**, 344–356.

Luo, Y., Sun, L., Zhu, Z., Ran, W., and Shen, Q. 2013. Identification and characterization of an antifungi *Fusarium oxysporum* f. sp. *cucumerium* protease from the *Bacillus subtilis* strain N7. *J. Microbiol.* **51**, 359–366.

Marimuthu, S., Ramamoorthy, V., Samiyappan, R., and Subbian, P. 2013. Intercropping system with combined application of *Azospirillum* and *Pseudomonas fluorescens* reduces root rot incidence caused by *Rhizoctonia bataticola* and increases seed cotton yield. *J. Phytopathol.* **161**, 405–411.

Moretti, M., Gilardi, G., Gullino, M.L., and Garibaldi, A. 2008. Biological control potential of *Achromobacter xylosoxydans* for suppressing Fusarium wilt of tomato. *Int. J. Bot.* **4**, 369–375.

Ohike, T., Makuni, K., Okanami, M., and Ano, T. 2013. Screening

of endophytic bacteria against fungal plant pathogens. *J. Environ. Sci.* **25**, 122–126.

Ordentlich, A., Elad, Y., and Chet, I. 1988. The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology* **78**, 84–88.

Park, W.P. 1995. Effect of starch sources on yulmoo kimchi fermentation. *Food Biotechnol.* **4**, 98–100.

Pathak, K.V. and Keharia, H. 2013. Characterization of fungal antagonistic bacilli isolated from aerial roots of banyan (*Ficus benghalensis*) using intact-cell MALDI-TOF mass spectrometry (ICMS). *J. Appl. Microbiol.* **114**, 1300–1310.

Pereira, P., Nesci, A., and Etcheverry, M. 2007. Effects of biocontrol agents on *Fusarium verticillioides* count and fumonisin content in the maize agroecosystem: impact on rhizospheric bacterial and fungal groups. *Biol. Control.* **42**, 281–287.

Prieto, P., Schiliro, E., Maldonado-Gonzalez, M.M., Valderrama, R., Barroso-Albarracin, J.B., and Mercado-Blanco, J. 2011. Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. *Microb. Ecol.* **62**, 435–445.

Pu, Y., Sun, L., Wang, Y., Qi, D., Chen, D., Liu, H., Xu, D., Deng, C., and Li, J. 2013. Modeling inhibitory activity of a novel antimicrobial peptide AMPNT-6 from *Bacillus subtilis* against *Vibrio parahaemolyticus* in shrimp under various environmental conditions. *Food. Control* **33**, 249–253.

Reinhold-Hurek, B. and Hurek, T. 2011. Living inside plants: bacterial endophytes. *Curr. Opin. Plant. Biol.* **14**, 435–443.

Sakiyama, C.C.H., Paula, E.M., Pereira, P.C., Borges, A.C., and Silva, D.O. 2001. Characterization of pectin lyase produced by an endophytic strain isolated from coffee Cherries. *Lett. Appl. Microbiol.* **33**, 117–121.

Sambrook, J. and Russel, D.W. 2001. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, N.Y., USA.

Santoyo, G., Orozco-Mosqueda, M.D.C., and Govindappa, M. 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. *Biocontrol. Sci. Technol.* **22**, 855–872.

Schisler, D.A., Slininger, P.J., Behle, R.W., and Jackson, M.A. 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* **94**, 1267–1271.

Seghers, D., Wittebolle, L., Top, E.M., Verstraete, W., and Siciliano, S.D. 2004. Impact of agricultural practice on the *Zea mays* L. endophytic community. *Appl. Environ. Microbiol.* **70**, 1475–1482.

Seo, W.T., Lim, W.J., Kim, E.J., Yun, H.D., Lee, Y.H., and Cho, K.M. 2010. Endophytic bacterial diversity in the young raddish and their antimicrobial activity against pathogens. *J. Korean Soc. Appl. Biol. Chem.* **53**, 493–503.

Sessitsch, A., Reiter, B., and Berg, G. 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth promoting and antagonistic abilities. *Can. J. Microbiol.* **50**, 239–249.

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

Sturz, A.V. 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.

Takahashi, M., Kita, Y., Kusaka, K., Mizuno, A., and Goto-Yamamoto, N. 2015. Evaluation of microbial diversity in the pilot-scale beer brewing process by culturedependent and culture-independent method. *J. Appl. Microbiol.* **118**, 454–469.

Verma, S.C., Ladha, J.K., and Tripathi, A.K. 2001. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *J. Biotechnol.* **81**, 127–141.