The Journal of Microbiology, June 2005, p.219-227 Vol. 43, No. 3
Copyright © 2005, The Microbiological Society of Korea

Isolation and Characterization of Bacteria Associated with Two Sand Dune Plant Species, *Calystegia soldanella* and *Elymus mollis*

Myung Soo Park[1], Se Ra Jung[1], Myoung Sook Lee[1], Kyoung Ok Kim[1], Jin Ok Do[1], Kang Hyun Lee[1], Seung Bum Kim[2,*] and Kyung Sook Bae[1]

[1]*Korea Research Institute of Bioscience and Biotechnology, 52 Oun-dong, Yusong, Daejeon 305-333, Republic of Korea*
[2]*Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, Daejeon 305-764, Republic of Korea*

(Received March 4, 2005 / Accepted April 12, 2005)

Little is known about the bacterial communities associated with the plants inhabiting sand dune ecosystems. In this study, the bacterial populations associated with two major sand dune plant species, *Calystegia soldanella* (beach morning glory) and *Elymus mollis* (wild rye), growing along the costal areas in Tae-An, Chungnam Province, were analyzed using a culture-dependent approach. A total of 212 bacteria were isolated from the root and rhizosphere samples of the two plants, and subjected to further analysis. Based on the analysis of the 16S rDNA sequences, all the bacterial isolates were classified into six major phyla of the domain *Bacteria*. Significant differences were observed between the two plant species, and also between the rhizospheric and root endophytic communities. The isolates from the rhizosphere of the two plant species were assigned to 27 different established genera, and the root endophytic bacteria were assigned to 21. Members of the phylum *Gammaproteobacteria*, notably the *Pseudomonas* species, comprised the majority of both the rhizospheric and endophytic bacteria, followed by members of *Bacteroidetes* and *Firmicutes* in the rhizosphere and *Alphaproteobacteria* and *Bacteroidetes* in the root. A number of isolates were recognized as potentially novel bacterial taxa. Fifteen out of 27 bacterial genera were commonly found in the rhizosphere of both plants, which was comparable to 3 out of 21 common genera in the root, implying the host specificity for endophytic populations. This study of the diversity of culturable rhizospheric and endophytic bacteria has provided the basis for further investigation aimed at the selection of microbes for the facilitation of plant growth.

Key words: rhizobacteria, endophytic bacteria, sand dune plant, 16S rDNA sequence, *Pseudomonas*

The interactions between plants and bacteria help plants to settle in ecosystem restoration processes (Glick, 1995). Plant-associated bacteria may increase the ability of plants to utilize nutrients from the soil by increasing root development, nitrate uptake or solubilizing phosphorus, and to control soil-borne pathogens (Smith and Read, 1997; Whipps, 2001). Strains of *Pseudomonas* and *Bacillus* have been shown to promote plant growth by producing phytohomones and solubilizing phosphates (Song *et al.*, 2003). Wei *et al.* (1996) reported that plant growth promoting rhizobacteria (PGPR) induced systemic resistance to cucumber disease and increased plant growth. Plant health and sustainability are dependent on the vast soil communities that contain bacterial populations (Torsvik *et al.*, 1990). Plants are known to alter the composition of microbial communities associated with their roots (Grayston *et al.*, 1996; Marschner *et al.*, 2001). Lemanceau *et al.* (1995) found that the soil-borne population of fluorescent pseudomonads in the rhizosphere of flax and tomato clearly differed from that in the bulk soil. In order to understand the effects of plant-bacteria interactions, it is essential to study the bacterial diversity associated with plants, and there have actually been a number of studies characterizing the structures and functions of rhizosphere and root bacterial communities (Hallmann *et al.*, 1997; Mahaffee and Kloepper, 1997; Maloney *et al.*, 1997; Germida *et al.*, 1998).

Plant communities in sand dunes are controlled by the interaction between biotic and physico-chemical components of the sand matrix (Read, 1989). Interactions with microbes appear crucial in obtaining inorganic nutrients or growth-influencing substances. In addition, human activities may also be an important factor, as they will certainly affect the vegetation as well as plant-microbe interactions. Plant-microbe symbioses have been exploited in

* To whom correspondence should be addressed.
(Tel) 82-42-821-6412; (Fax) 82-42-822-7367
(E-mail) sbk01@cnu.ac.kr

programs of sand dune restoration. Arbuscular mycorrhizal fungi are important to some sand dune plants used in restoration projects of coastal sand ecosystems (Sylvia and Burks, 1988). Dalton *et al.* (2004) suggested that the nitrogen-fixing bacteria isolated from the rhizosphere and root of *Ammophila arenaria* may contribute to the prolific success of these plants in nutrient-poor sand. Despite the important role played by bacterial diversity in sand dune plant communities, little is known on the distribution and abundance of root or rhizosphere associated bacteria.

This study is the first report on the diversity of culturable bacteria associated with the two major sand dune plant species, *Calystegia soldanella* (beach morning glory) and *Elymus mollis* (wild rye), which are found as the dominant plant species along the coastal sand dune areas in Tae-An, Chungnam Province. The outcome of this study will form the basis for the selection of symbiotic bacteria that can be utilized for the facilitation of plant growth, and thus for the restoration of vegetation in this sand dune ecosystem.

Materials and Methods

Sampling of plants and rhizosphere soil

Plants and soil samples were collected from four coastal sand dune areas of the Tae-An, Chungnam Province, in August 2003 (Table 1). These areas included actively growing zones of *C. soldanella* and *E. mollis,* which vigorously stand on the seaward dune faces. Three samples of *C. soldanella* were taken from Baramarae, Sambong and Shindu, and two samples of *E. mollis* from Hagam and Sambong, respectively.

Isolation of bacteria

The roots were separated from the soil and washed in tap water, surface-sterilized with ethanol for 1 min, 3% NaOCl for 3 min and then rinsed in sterile distilled water. The surface-sterilized root mass was pulverized in a ceramic mortar, and homogenized with sea sand. The rhizosphere soils recovered after root removal were also collected for isolation of bacteria.

Table 1. Distribution of representative bacterial taxa in the rhizosphere of sand dune plants†

Affiliation Family	Genus	Species	*C. soldanella*			*E. mollis*	
			Baramarae (35)	Sambong (19)	Shindu (23)	Sambong (32)	Hagam (31)
Micrococcaceae	*Arthrobacter*	*histidinolovorans*	1			4	
		nitroguajacolicus		1	2	1	
Microbacteriaceae	*Curtobacterium*	*citreum*	2				
	Microbacterium	*testaceum*	2				
Bacillaceae	*Exiguobacterium*	*acetylicum*					3
Flavobacteriaceae	*Chryseobacterium*	*defluvii*					2
		indoltheticum	2				
		proteolyticum			3		1
		scophthalmum				2	
	Flavobacterium	*saccharophilum*					3
Sphingobacteriaceae	*Pedobacter*	*heparinus*			2	1	
Moraxellaceae	*Acinetobacter*	*calcoaceticus*	2	2		3	
Enterobacteriaceae	*Enterobacter*	*asburiae*		2			
		dissolvens					2
		hormaechei			4		
	Klebsiella	*pneumoniae*					6
		oxytoca					3
	Pantoea	*agglomerans*	2				
Pseudomonadaceae	*Pseudomonas*	*lini*		7	4		4
		montelli	2				
		oleovorans	5				
		parafulva				8	
		putida	2			1	
Xanthomonadaceae	*Stenotrophomonas*	*maltophilia*				2	
		rhizophilia		1	3		

†Numbers indicate strains assigned to each species, and those in parentheses are the total numbers of isolates.

Table 2. Distribution of representative bacterial taxa in the root of sand dune plants†

Affiliation Family	Genus	Species	*C. soldanella*			*E. mollis*	
			Baramarae (5)	Sambong (31)	Shindu (16)	Sambong (3)	Hagam (17)
Microbacteriaceae	*Leifsonia*	*poae*			2		
	Microbacterium	*foliorum*			3		
		oxydans		3			
Paenibacillaceae	*Brevibacillus*	*brevis*		4			
	Paenibacillus	*illinoisensis*		3	2		
Flavobacteriaceae	*Chryseobacterium*	*joostei*		2			
		proteolyticum			2		2
Rhizobiaceae	*Agrobacterium*	*larrymoorei*	2				
	Rhizobium	*huatlense*				2	
Moraxellaceae	*Acinetobacter*	*calcoaceticus*		6			
Aeromonadaceae	*Aeromonas*	*popoffii*					2
Pseudomonadaceae	*Pseudomonas*	*graminis*			2		
		koreensis		4			
		plecoglossicida					8
Chromatiaceae	*Rheinheimera*	*pacifica*					2
Xanthomonadaceae	*Rhodanobacter*	*lindaniclasticus*			2		

†See the footnote for Table 1.

Root and soil dilutions were prepared by suspending 5 g of homogenized root and rhizosphere soils in 50 ml of phosphate-buffered saline (PBS; containing 8 g/l NaCl, 0.2 g/l KCl, and 1.4 g/l Na_2HPO_4) in 125 ml Erlenmeyer flasks. The flasks were incubated in an orbital shaker at 150 rpm for 1 h. One ml of soil suspension was added to 9 ml portions of PBS in glass tubes. Dilution series were prepared by further transfers, and used as inocula for plate count experiments. The 100 μl aliquots from different dilutions were transferred and spread onto R2A and nutrient agar plates. In all cultivation experiments, the agar plates were incubated in the dark for 2 days at 25°C. Based on the colony characteristics, single colonies were selected and stored in 15% glycerol at -80°C for later characterization and identification.

DNA extraction, PCR amplification and sequencing

DNA was extracted from the bacteria using the following protocol. The bacterial cells were taken into an Eppendorf tube containing 100 μl of STES buffer (500 mM NaCl, 200 mM Tris-HCl (pH 7.6) and 10 mM EDTA, 1% SDS) and glass beads. The mixture was vortexed for 5 min using a TOMY micro tube mixer (TOMY, Japan) and then 200 μl of TE buffer (pH 8.0) added. DNA was purified through phenol/chloroform/isoamyl alcohol (25:24:1) extraction. RNA was removed by RNase A treatment at 37°C for 3 h. The purified DNA was precipitated with 0.1 volumes of 3 M sodium acetate and 2 volumes of cold 95% ethanol, and then centrifuged at 12,000 g for 20 min at room temperature. The supernatant was removed, the pellet washed with 70% ethanol, dried in air and then resuspended in 50 μl of TE buffer. The resuspended DNA was stored at -20°C until required. The 16S rRNA genes were PCR-amplified with the bacterial universal primers, 27F and 1492R (Lane, 1991), and purified for sequencing.

Restriction fragment length polymorphism (RFLP) analysis

The amplified 16S rDNAs were subjected to RFLP analysis for their rapid characterization. The 10 μl of each PCR product was digested overnight with 3 units of *Hae*III at 37°C, and then separated on 4% agarose gel. The strains were grouped according to their RFLP patterns, and selected strains, with representative patterns, sequenced. The group of strains that yielded the same RFLP pattern was defined as an operational taxonomic unit (OTU).

Phylogenetic analysis

Purified double stranded PCR fragments were directly sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, USA), according to the manufacturer's instructions. The same forward primer for the PCR amplification was also used for the partial sequencing. The gel electrophoresis and data collection were performed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA.). The sequences were compared with the 16S rDNA sequences available in the public databases from a BLAST search, and identified to the generic level.

The sequences generated from the materials in this study, and retrieved from GenBank, were initially aligned using the CLUSTAL X program (Thompson *et al.*, 1997), and the alignment then refined manually using version 3.0 of the PHYDIT program (Chun, 1995; available at http://plaza.snu.ac.kr~jchun/phydit). Ambiguously aligned regions were excluded from subsequent analyses. A neighbor-joining tree was inferred with Kimura's 2-parameter distance model (Kimura, 1980) using the PHYLIP 3.57c package (Felsenstein, 1985). A bootstrap analysis, using 1,000 replications, was performed to assess the relative stability of the branches.

Results

Identification of bacterial isolates

A total of 212 bacterial strains were isolated and maintained; 77 and 52 from the rhizosphere and root of *C. soldanella*, and 63 and 20 from those of *E. mollis*, respectively (Tables 1 and 2). The bacterial isolates were initially grouped according to the RFLP patterns of their 16S rDNA, from which at least 28 and 30 different patterns among the rhizobacteria and root bacterial endophytes, respectively, were recognized (data not shown). Representative strains were chosen from each pattern,

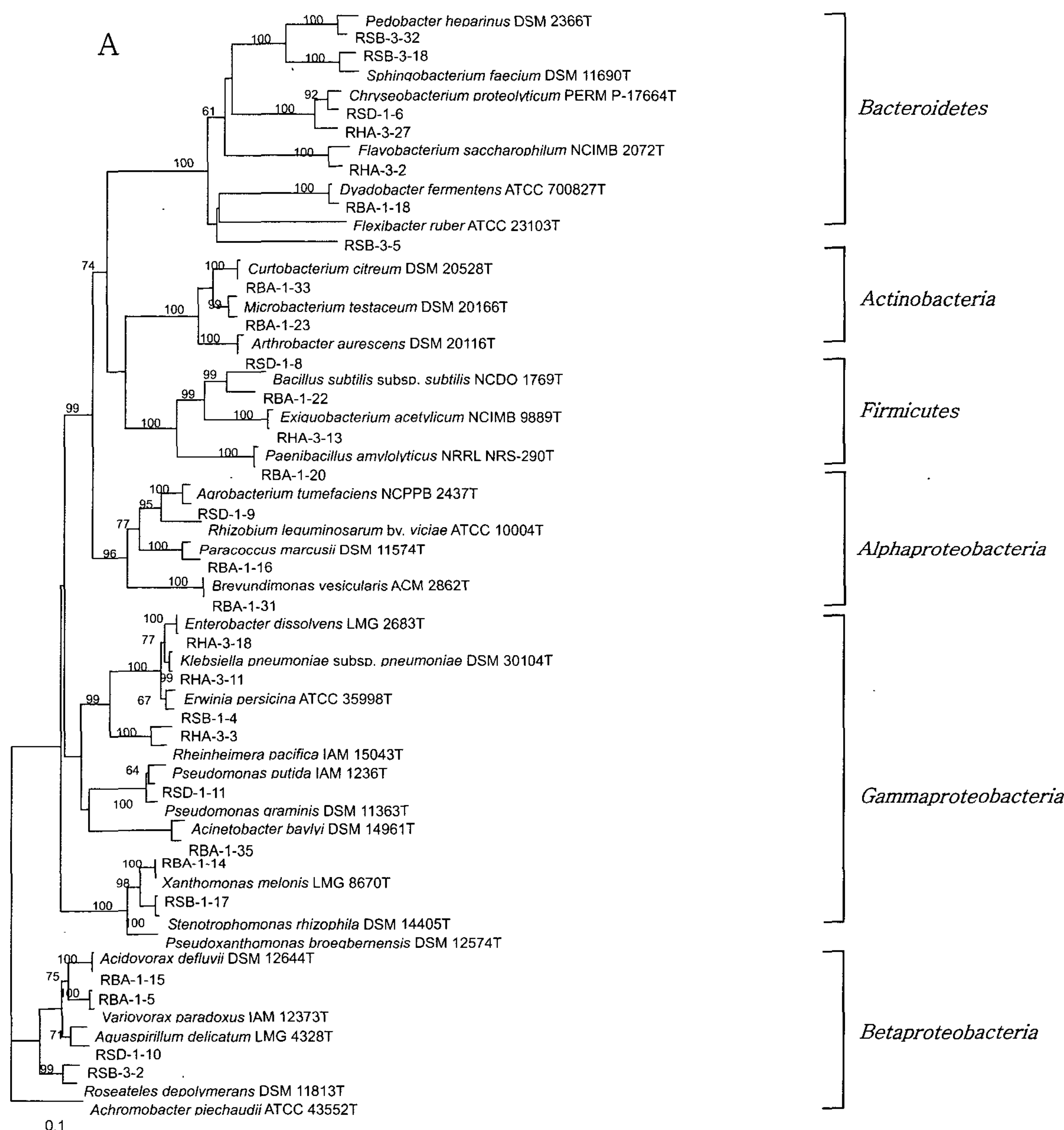

Fig. 1. Phylogenetic relationships among bacterial isolates from the rhizosphere (A) and roots (B) of *Calystegia soldanella* and *Elymus mollis* based on the partial 16S rDNA sequences. The trees were obtained by the neighbor-joining method using Kimura's two-parameter distance model. The numbers above each branch indicate bootstrap values of distance. The bootstrap values were obtained after a bootstrap test with 1000 replications. The bar represents 0.1 substitutions per site.

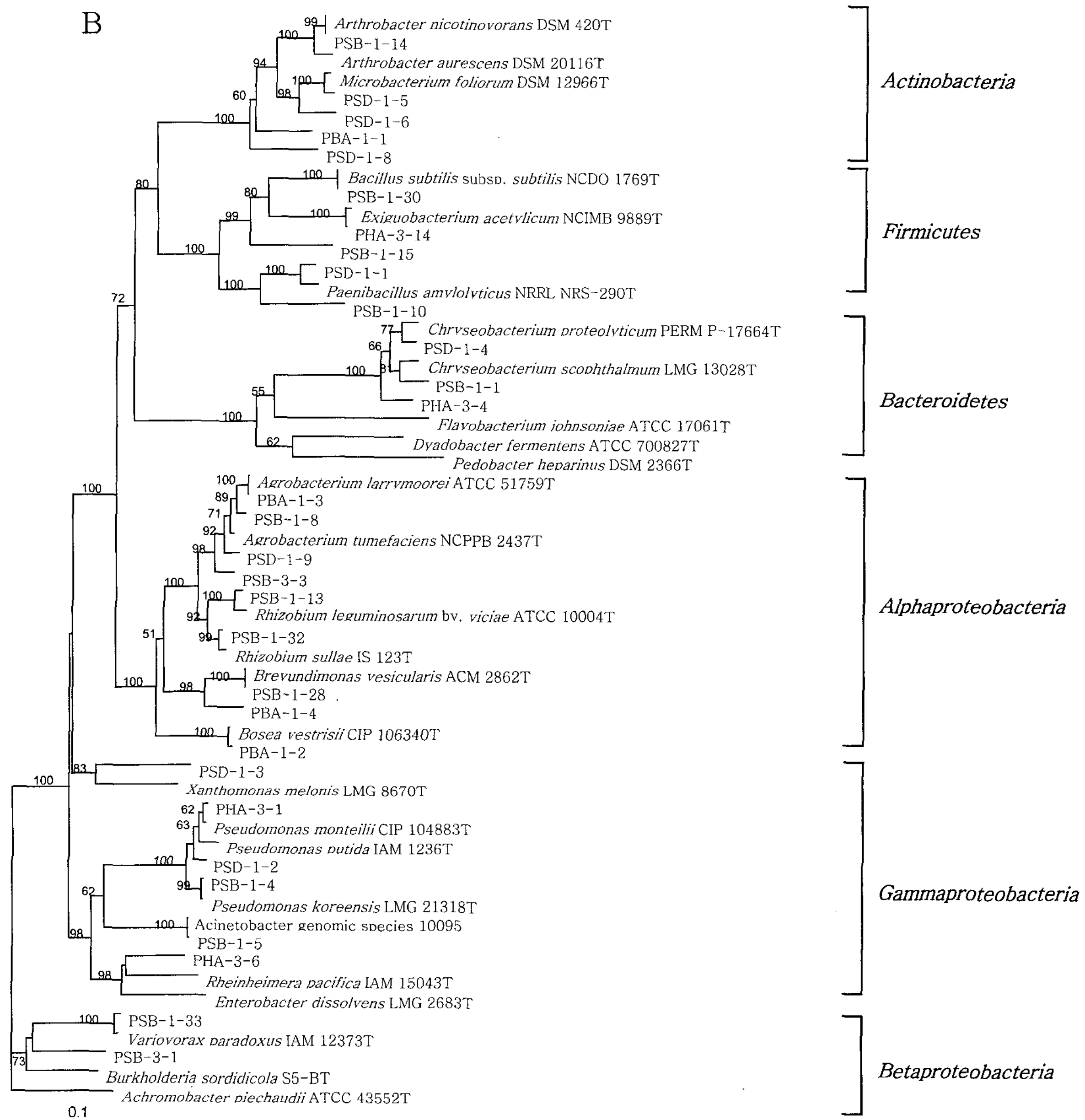

Fig. 1. Continued.

partially sequenced and identified to the species or genus level (Table 2). Strains yielding the same pattern were considered a single OTU, which was subsequently confirmed by sequencing several strains with the same pattern. A total of 104 strains were sequenced, and their partial 16S rDNA sequences deposited in the GenBank, under the accession numbers AY822478 ~ AY822581. The phylogenetic relationships among representative isolates are illustrated in Fig. 1. All the bacterial isolates were assigned to six phyla within the domain *Bacteria*, namely *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes* (Fig. 2).

Analysis of rhizospheric bacterial communities

The rhizobacterial isolates of *Calystegia soldanella* and *Elymus mollis* were represented by six phyla, with the majority (60%) of the isolates falling within the proteobacterial groups (Figs. 1A and 2). The members of phylum *Gammaproteobacteria* were predominant (59.2% of total isolates in *C. soldanella* and 46.6% in *E. mollis*, respectively), of which the genus *Pseudomonas* was the major taxon (33.8 and 19.0% of total isolates, respectively). Members of the phylum *Bacterodetes* were the second most abundant group (14.1 and 22.4%, respectively), made up largely of *Chryseobacterium* (8.5 and 8.6%, respectively). *Actinobacteria*, including *Arthrobacter*, *Microbac-*

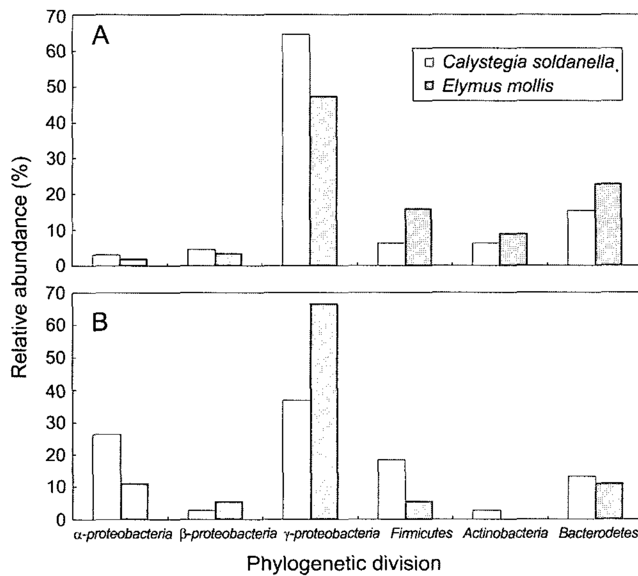

Fig. 2. The relative frequency of bacterial isolates belonging to different phylogenetic groups in the rhizosphere (A) and roots (B) of *Calystegia soldanella* and *Elymus mollis*. Phylogenetic assignment was determined by sequence analysis of the 16S rRNA gene.

terium and *Curtobacterium*, all belonging to the pleomorphic coccoid group *Micrococcineae*, accounted for 14.1 and 10.3% in *C. soldanella* and *E. mollis*, respectively, and *Firmicutes*, including *Bacillus*, *Brevibacillus* and *Exiguobacterium*, accounted for 5.6 and 15.5%, respectively.

All the bacterial isolates were assigned to 28 genera, 21 of which were found from *C. soldanella* and 22 from *E. mollis*. The four bacterial genera, *Pseudomonas, Chryseobacterium, Arthrobacter* and *Enterobacter,* represented 49% of the isolates from the rhizosphere of the two plants. The distribution patterns of the major bacterial taxa differed between the two plant species (Table 1). For example, strains of *Curtobacterium* (4.2%) were isolated only from *C. soldanella,* but not from *E. mollis*. In contrast, *Klebsiella* (12.1%) and *Brevibacillus* (6.9%) were isolated only from *E. mollis*. However, no single species among these taxa was responsible for the dominance, and in fact no plant specific pattern was observed in either plant species. For example, different species of *Pseudomonas*, namely *P. oleovorans*, *P. lini* and *P. parafulva,* were the most abundant species in different cases, but were not plant specific.

Analysis of root endophytic bacterial communities

The phylogenetic analysis indicated that the isolates from the roots of *C. soldanella* and *E. mollis* were taxonomically diverse, and could also be grouped into 6 phyla (Figs. 1B and 2). The majority of isolates from the roots of the two plants were grouped within the three proteobacterial phyla (Fig. 2). Members of *Gammaproteobacteria* were predominant (30.4% in *C. soldanella* and 66.7% in *E. mollis*), and many of the isolates were again related to *Pseudomonas* (13.0 and 44.4%, respectively). Genera belonging to *Alphaproteobacteria* (21.7%) and *Actinobacteria* (19.6%) also comprised major taxa in *C. soldanella.* It was notable that a number of *Actinobacteria* strains were isolated from root of *C. soldanella,* but not from *E. mollis* (Fig. 1).

The root endophytic bacterial isolates were represented by 16 and 7 genera in *C. soldanella* and *E. mollis*, respectively. *Pseudomonas, Chryseobacterium* and *Rhizobium* were the only common genera isolated from both plant species (Table 2). In contrast to the rhizosphere samples, members of *Enterobacteriaceae* were not isolated from the root samples. *Agrobacterium* (10.9%), *Acinetobacter* (13.0%), *Paenibacillus* (13.0%) and *Microbacterium* (13.0%) were commonly isolated from *C. soldanella,* but not from *E. mollis*. *Aeromonas* (11.1%) and *Rheinheimera* (11.1%) were also isolated from *C. soldanella,* but not from *E. mollis.*

Comparative analysis between rhizospheric and root endophytic bacterial communities

The majority of rhizobacteria and root endophytic bacteria from both *C. soldanella* and *E. mollis* were proteobacteria, comprising 63% of the total isolates in the rhizosphere and 71% in the roots. The members of *Gammaproteobacteria* were the most abundant in all four cases (Tables 1 and 2). The *Alphaproteobacteria,* containing *Agrobacterium* and *Rhizobium,* were isolated more frequently from the roots than from the rhizosphere, while the *Gammaproteobacteria* and *Bacteroidetes* groups were isolated more frequently from the rhizosphere (Fig. 2).

The total bacterial isolates comprised 36 genera, among which 28 were present in the rhizosphere and 20 in the roots. There were obvious differences between the rhizosphere and root bacterial communities, and also between the two plant species. Lower bacterial diversity was observed in the roots compared to the rhizosphere. *Pseudomonas* and *Chryseobacterium* were the most common genera found in both the rhizosphere and roots of the two plants. Strains of *Enterobacter, Klebsiella, Stenotrophomonas* and *Flavobacterium,* found frequently in the rhizosphere, were not isolated from the roots. The common species found in both the rhizosphere and roots included *Acinetobacter calcoaceticus* and *Chryseobacterium proteolyticum*. It was notable that the species composition of *Pseudomonas* differed according to the plant species, sites and parts sampled.

Discussion

This study is part of an ongoing project, which includes the examination of the diversity of bacterial populations associated with the rhizosphere and roots of two major coastal sand dune plants, *C. soldanella* and *E. mollis,*

using a culture-dependent approach, as well as the collection of bacteria that could be used to facilitate plant growth. While there have been a few studies on the microbial diversity of terrestrial environments in Korea (Lee *et al.*, 2000; Park and Ka, 2003, Lim *et al.*, 2004), virtually no information is available for the microbial diversity associated with wild plants in domestic environments.

The cultivated bacterial isolates obtained through this study were represented by 36 known bacterial genera from the phyla *Alpha-*, *Beta-* and *Gammaproteobacteria*, as well as *Firmicutes, Actinobacteria* and *Bacteroidetes*. Members belonging to *Gammaproteobacteria* were predominant in most cases, with many of these isolates assigned to *Pseudomonas* in both the rhizosphere soils and roots of the two plants, whereas the composition of species was different according to the environment (Tables 1 and 2). For example, the rhizospheric *Pseudomonas* strains were phylogenetically related to *P. lini* and *P. oleovorans* (*C. soldanella*) or *P. parafulva* (*E. mollis*), whereas the endophytic strains were related to *P. graminis* and *P. koreensis* (*C. soldanella*) or *P. plecoglossicida* (*E. mollis*). *Chryseobacterium* was the next most common genus, and the species composition was again significantly different between samples. The rhizospheric *Chryseobacterium* strains were most closely related to *C. proteolyticum*, and the endophytic strains to either *C. proteolyticum* or *C. scophthalmum*. Based on the 16S rDNA analysis, a number of isolates could be recognized as potentially new species or higher taxa, as all of the listed strains showed lower than 97% 16S rDNA sequence similarities with any validly described species. Notably, strains assigned to the phylum *Bacteroidetes*, in particular those assigned to *Chryseobacterium*, comprised the majority of potentially novel taxa. Strain RSB-3-5 shared 81.5% partial 16S rDNA sequence similarity with the highest match, thus implying this strain may represent a novel phylogenetic lineage at the family or even a higher taxonomic level. Further taxonomic characterization may be necessary to examine the precise taxonomic positions of such isolates. The dominance of *Gammaproteobacteria*, particularly species of *Pseudomonas*, was in line with previous observations (Marilley and Aragno, 1999).

The bacterial communities associated with the rhizosphere and roots differed significantly between the two plant species. *Agrobacterium* spp. (5.1%) were isolated from *C. soldanella,* but not from *E. mollis*. In contrast, *Klebsiella* (9.2%) and *Brevibacillus* (5.2%) were isolated only from the latter. The differences in the microbial communities between plants species have also been observed in many other studies (Germida *et al.*, 1998; Grayston *et al.*, 1998; Miethling *et al.*, 2000), which are likely to be due to differences in the amount and composition of root exudates as well as the root cell components at the root tip and in the mature root zone (Whipps, 1990; Grayston *et al.*, 1996; Maloney *et al.*, 1997; Merbach *et al.*, 1999). Root exudates are widely reported to control rhizosphere populations. The low molecular mass organic acids, known as root exudates, in the rhizosphere of wheat and flax differ significantly between cultivars (Cieslinski *et al.*, 1997). Marschner *et al.* (2001) observed that the bacterial community composition in the rhizosphere is affected by a complex interaction between plant species, soil type and root zone.

Diverse *Pseudomonas* strains have been found in rhizosphere and roots, but the bacterial community in the root of *C. soldanella* was not dominated by any one genus or species. *Acinetobacter*, *Pseudomonas*, *Paenibacillus*, *Microbacterium*, *Agrobacterium* and *Chryseobacterium* were commonly isolated in the root of *C. soldanella*, each comprising $11 \sim 13\%$ of the total isolates. Lilley *et al.* (1996) also found that the community composition in the root of sugar beet was not dominated by any single genus. Pseudomonads are considered as important constituents in the root-associated microbial community, and their ability to colonize the root surface, preventing the development of plant pathogens and improving plant growth, is well known. However, the result from this study suggests that other bacterial taxa, such as *Chryseobacterium*, *Acinetobacter*, *Arthrobacter* and *Microbacterium*, can also be important and, thus have potential for their application to plant growth facilitation.

The bacterial communities of the rhizosphere and roots of the two plants were distinct in structure at the genus level, but shared some common groups. Relatively low numbers of isolates and also taxa were observed in the root compared to the rhizosphere, while the endophytic communities between the plant species varied significantly. This reduced diversity was in line with the observations of the dominating roles of plants in the control of the endophytic bacterial community composition (Germida *et al.*, 1998; Siciliano *et al.*, 1999). The bacteria associated with roots can protect plant growth through the biocontrol of plant pathogens, stimulate plant growth through the production phytohormones and enhancement of the availability of nutrient, and induce systemic resistance to disease (Bakker and Schippers, 1987; Jacobson *et al.*, 1994; Chanway, 1996; Wei *et al.*, 1996; Van Loon *et al.*, 1998; Singh *et al.*, 2003; Lucy *et al.*, 2004; Mantelin, and Touraine, 2004).

This study will provide basic information on the diversity of bacteria associated with major plant species inhabiting the coastal sand dune area of Tae-an, Korea. While the study of bacterial diversity, using a culture-dependent approach, has its own limitation, its main advantage is that a number of bacteria can actually be isolated and characterized for further study, including plant growth promoting activity and development of strategies for the restoration of vegetation in such ecosystems. The sustainability of sand dune ecosystems will be dependent on the ability of plants to acquire soil

nutrients and water by an effective method, and also on the resistance of sand dune plants to phytopathogens. The artificial introduction of plants to such environments will also be greatly influenced by the root-associated bacterial community, and therefore, it is essential to gain greater understand of the plant-microbe interaction within ecosystems. The bacteria isolated in this study are considered to represent the culturable diversity of healthy plants, and thus potentially beneficial to the growth and survival of plants in that specific ecosystem. Future work will need to include tests of the ability to promote plant growth, control activities of plant pathogenic microorganisms and also to compare the diversity using culture-independent approaches.

Acknowledgements

This research was undertaken with the support from the Core Environmental Technology Development Project for the Next Generation (Eco-Technopia-21), sponsored by the Ministry of Environment, Republic of Korea.

References

Bakker, A.W. and P. Schippers. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Psudomonas* spp.-mediated plant growth-stimulation. *Soil Biol. Biochem.* 19, 451-457.

Chanway, C.P., L.M. Nelson, and F.B. Holl. 1988. Cultivar-specific growth promotion of spring wheat (*Triticum aestivum* L.) by coexistent *Bacillus* species. *Can. J. Microbiol.* 34, 925-929.

Chun, J. 1995. Computer-assisted classification and identification of actinomycetes. Ph. D. thesis, University of Newcastle, Newcastle upon Tyne, UK.

Cieslinski, G., K.C.J. Van-Rees, A.M. Szmigielska, and P.M. Huang. 1997. Low molecular weight organic acids released from roots of durum wheat and flax into sterile nutrient solutions. *J. Plant Nutr.* 20, 753-764.

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

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39, 783-791.

Germida, J.J., S.D. Siciliano, J.R. de Freitas, and A.M. Seib. 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.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* 41, 109-117.

Grayston, S.J., D. Vaughan, and D. Jones. 1996. Rhizosphere carbon flow in tree, in comparison with annual plants, the importance of root exudation and its impact on microbial diversity and nutrient availability. *Appl. Soil Ecol.* 5, 29-56.

Grayston, S.J., S. Wang, C.D. Campbell, and A.C. Edwards. 1998. Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol. Biochem.* 30, 369-378.

Hallmann, J., A. Quadt-Hallmann, W.F. Mahaffee, and J.W. Kloepper 1997. Bacteria endophytes in agricultural crops. *Can. J. Microbiol.* 43, 895-914.

Jacobson, C.B., J.J. Pasternak, and B.R. Glick. 1994. Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth promoting rhizobacterium *Psudomonas putida* GR12-2. *Can. J. Microbiol.* 40, 1019-1025.

Jeon, J.-S., S.-S. Lee, H.-Y. Kim, T.-S. Ahn, and H.-G. Song. 2003. Plant growth promotion in soil by some inoculated microorganisms. *J. Microbiol.* 41, 271-276.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequence. *J. Mol. Evol.* 16, 111-120.

Lane, D.J. 1991. 16S/23S rRNA sequencing, p. 115-175. *In* E. Stackebrandt and M. Goodfellow (eds.), Nucleic Acid Techniques in Bacterial Systematics. John Weley & Sons, Chichester, UK.

Lee, D.-H., S.-A. Noh, and C.-K. Kim. 2000. Development of molecular biological methods to analyze bacterial species diversity in freshwater and soil ecosystems. *J. Microbiol.* 38, 11-17.

Lemanceau, P., T. Corbererand, L. Gardan, X. Latour, G. Laguerre, J-M. Boeufgras, and C. Alabouvette. 1995. Effect of two plant species, flax (*Linum usitatissinum*) and tomato (*Lycopersicon esculentum* Mill) on the diversity of soilborne populations of fluorescent *Pseudomonas*. *Appl. Environ. Microbiol.* 61, 1004-1012.

Lilley, A.K., J.C. Fry, M.J. Bailey, and M.J. Day. 1996. Comparison of aerobic heterotrophic taxa isolated from four root domains of mature sugar beet (*Beta vulgaris*). *FEMS Microbiol. Ecol.* 21, 231-242.

Lim, J.-S., M.-K. Jung, M.-S. Kim, J.-H. Ahn, and J.-O. Ka. 2004. Genetic and phenotypic diversity of (R/S)-mecoprop [2-(2-methyl-4-chlorophenoxy)propionic acid]-degrading bacterial isolated from soils. *J. Microbiol.* 42, 87-93.

Lucy, M., E. Reed, and B.R. Glick. 2004. Applications of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek.* 86, 1-25.

Mahaffee, W.F. and J.W. Kloepper. 1997. Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). *Microb. Ecol.* 34, 210-223.

Maloney, P.E., A.H.C. Van Bruggen, and S. Hu. 1997. Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizosphere and in bulk soil. *Microb. Ecol.* 34, 109-117.

Mantelin, S. and B. Touraine. 2004. Plant growth-promoting bacteria and nitrate availability; impacts on root development and nitrate uptake. *J. Exp. Bot.* 55, 27-34.

Marilley, L. and M. Aragno. 1999. Phylogenetic diversity of bacterial communities differing in degree of proximity of *Lolium perenne* and *Trifolium repens* roots. *Appl. Soil Ecol.* 13, 127-136.

Marschner, P., C.H. Yang, R. Lieberei, and D.E. Crowley. 2001. Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biol. Biochem.* 33, 1437-1445.

Merbach, W., E. Mirus, G. Knof, R. Remus, S. Ruppel, R. Russow, A. Gransee, and J. Schulze. 1999. Release of carbon and nitrogen compounds by plant roots and their possible ecological importance. *J. Plant Nutr. Soil Sci.* 162, 373-383.

Miethling, R., G. Weiland, H. Backhaus, and C.C. Tebbe. 2000. Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with *Sinorhizobium meliloti* L 33. *Microb. Ecol.* 40, 43-56.

Park, H.-D. and J.-O. Ka. 2003. Genetic and phenotypic diversity of dichloroprop-degrading bacteria isolated from soils. *J. Microbiol.* 41, 7-15.

Read, D.J. 1998. Mycorrhizas and nutrient cycling in sand dune ecosystems. Coastal Sand Dunes, vol. 96, p. 89-110. *In* C.H. Gimmingham, W. Ritchie, B.B. Willetts and A.J. Willis (eds.), Proceedings of Symposium. The Royal Society of Edinburgh, Edinburgh.

Siciliano, S.D. and J.J. Germida. 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.

Singh, U.P., B.K. Sarma, and D.P. Singh. 2003. Effect of plant-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cier arietinum*). *Curr. Microbiol.* 46, 131-140.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, San Diego, California, USA.

Sylvia, D.M. and N.J. Burks. 1988. Selection of a vesicular-arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata*. *Mycologia* 80, 565-568.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.

Torsvik, V., J. Gokøyr, and F.L. Daae. 1990. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* 56, 782-787.

Van Loon, L.C., P.A. Bakker, and C.M. Pieterse. 1998. Systemic resistance induced by rhizosphere bacteria. *Ann. Rev. Phytopathol.* 36, 453-483.

Wei, G.J., W. Kloepper, and S. Tuzun. 1996. Induced systemic resistance to cucumber disease and increased plant growth by plant growth-promoting rhizobacteria under field condition. *Phytopathology* 86, 221-224.

Whipps, J.M. 1990. Carbon economy, p. 59-97. *In* J.M. Lynch (ed.), The Rhizosphere. John Wiley & Sons, Essex, UK.

Whipps, J.M. 2001. Microbial interaction and biocontrol in the rhizosphere. *J. Exp. Bot.* 52, 487-511.