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Research Paper

A primary assessment of the endophytic bacterial community in a xerophilous moss (*Grimmia montana*) using molecular method and cultivated isolates

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Abstract

Investigating the endophytic bacterial community in special moss species is fundamental to understanding the microbial-plant interactions and discovering the bacteria with stresses tolerance. Thus, the community structure of endophytic bacteria in the xerophilous moss *Grimmia montana* were estimated using a 16S rDNA library and traditional cultivation methods. In total, 212 sequences derived from the 16S rDNA library were used to assess the bacterial diversity. Sequence alignment showed that the endophytes were assigned to 54 genera in 4 phyla (Proteobacteria, Firmicutes, Actinobacteria and *Cytophaga/Flexibacter/Bacteroids*). Of them, the dominant phyla were Proteobacteria (45.9%) and Firmicutes (27.6%), the most abundant genera included *Acinetobacter*, *Aeromonas*, *Enterobacter*, *Leclercia*, *Microvirga*, *Pseudomonas*, *Rhizobium, Planococcus, Paenisporosarcina and Planomicrobium*. In addition, a total of 14 species belonging to 8 genera in 3 phyla (Proteobacteria, Firmicutes, Actinobacteria) were isolated, *Curtobacterium, Massilia, Pseudomonas* and *Sphingomonas* were the dominant genera. Although some of the genera isolated were inconsistent with those detected by molecular method, both of two methods proved that many different endophytic bacteria coexist in *G. montana*. According to the potential functional analyses of these bacteria, some species are known to have possible beneficial effects on hosts, but whether this is the case in *G. montana* needs to be confirmed.

Key words: bacterial diversity, endophytes, moss, molecular method, cultivated isolates.

Introduction

In plant-endophyte interactions, plants provide nutrients and residency for the bacteria, while the bacteria in exchange directly or indirectly improve plant growth and health (Mastretta *et al.*, 2006). Once inside the plant, endophytes either reside in specific plant tissues such as the root cortex or the xylem, or colonize the plant systematically by transport through the vascular system or the apoplast (Quadt-Hallmann *et al.*, 1997). Of the nearly 300 000 plant species on earth, each species is host to one or more species of endophytes (Strobel *et al.*, 2004). The complete description of endophytic species has only been enumerated and characterized for a handful of plant species, and the majority of these are common higher plants. Few studies have examined the endophytes of bryophytes, which represent the simplest extant land plants and have been classified by prominent bryologists as "living fossils" (Hornschuh *et al.*, 2002). Consequently, the opportunity to find new and beneficial endophytic microorganisms among the diversity of plants in different ecosystems is considerable.

The mosses, one kind of bryophytes, are a diverse group of land plants that usually colonize habitats with either moist or extremely variable conditions. One of their most important features is their life cycle, which involves alteration between a diploid sporophyte and a dominant free-living haploid gametophyte generation (Opelt and Berg, 2004). Mosses are unique host plants for microorganisms in numerous ways. For example, the small size of mosses results in limited availability of the substratum. In addition, most mosses display an extraordinarily high tolerance to extreme desiccation and can resume normal metabolism very rapidly after rehydration. Hence, successful microbial colonization requires adaptation to these special

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conditions (DoÈbbeler, 1997). Analysis of the epiphytes on the gametophyte of *Funaria hygrometrica* detected numerous bacterial species on the surface of the phylloid. Among these species, two *Methylobacterium* strains were found to be able to simulate the well-known effect of cytokinin application on bud formation in *Funaria* protonema and they also promoted the growth of protonemal filaments (Hornschuh *et al.*, 2002). Endophytic methanotrophic bacteria were also found in the hyaline cells and on the stem leaves of *Sphagnum* mosses; here, they provided carbon for photosynthesis via in situ oxidation of methane to carbon dioxide (Raghoebarsing *et al.*, 2005).

Opelt and Berg (2004) isolated and identified many antagonistic bacteria associated with three moss species (*Tortula ruralis*, *Aulacomnium palustre* and *Sphagnum rubellum*) in the nutrient-poor habitats of the Baltic Sea Coast in Germany. These species belong to nine different genera, among which *Burkholderia*, *Pseudomonas* and *Serratia* were dominant, but the richness and diversity of antagonistic species were moss species-dependent, and the highest number of species with antagonistic activity was isolated from *S. rubellum*. Another study examined the function and diversity of bacterial species associated with two *Sphagnum* species (*S. fallax* and *S. magellanicum*) that grow in a temperate mire ecosystem. Species belonging to the genus *Burkholderia* were predominant in *Sphagnum* species and this genus was possibly involved in antagonism/pathogen defense and nitrogen-fixation. The authors concluded that *Sphagnum* is a reservoir for powerful and extraordinary antagonists and potentially facultative human pathogens (Opelt *et al.*, 2007). Thus, thorough research on the bacteria associated with other mosses in different niches would be also useful in discovering bacterial resources and helpful in understanding the interactions between mosses and their associated microbes.

Grimmia montana is a xerophilous moss, and has a high tolerance to drought, cold and UV radiation (Yi and Liu, 2007), and can often be found growing in extreme environments. It always lives under extreme desiccation conditions and can resume normal metabolism very rapidly after rehydration. In this paper, our aim is to study the diversity and community structure of its endophytes using 16S rDNA library and culture-dependent approaches, and hope to make a well known on the interactions between endophytes and *G. montana* and try to find some bacterial resources with the strong tolerance to the stresses.

Material and Methods

Sampling and surface disinfection

Grimmia montana were sampled from the surface of one large stone in Beijing Songshan National Nature Reserve located at an altitude of 890 m, at N: 40°31'00.45" by E:115°49'33.20" on the 19th of April, 2011. About 3 g of plant material, approximately more than one thousand of entire plants was collected after absorbing enough water, and then mixed together and immediately transported to the laboratory for surface disinfection as described previously (Li *et al.*, 2010). The plants were first washed many times with tap water to remove attached substratum. Subsequently, they were immersed in 70% ethanol for 3 min, washed with 15% sodium hypochlorite solution for 10 min, rinsed three times with 70% ethanol for 30 s, and finally washed five times with sterile distilled water. To confirm that the disinfection process was successful, aliquots of the sterile distilled water in the final rinse were used to determine the results of surface disinfection. Bacteria were cultivated by setting 100 μL of the final rinse on R2A and TSA medium plates, and then examining the plates for bacterial growth after incubation at 28 °C for 3 days. Molecular detection of bacterial species was accomplished by 16S rRNA gene PCR detection based on the primers 799f (5'-AACAGGATTAGATACCCTG-3') and 1492r (5'-GGTTACCTTGTTACGACTT-3') (Chelius and Triplett, 2001) using the final rinse as template. The 50 μL PCR reaction mixture contained 5 μL of the final rinse, 5 μL 10x Taq reaction buffer (including 1.5 mM $MgCl_2$), 10 pmol of each primer, 200 μM each dNTP, and 1.5 U of Taq DNA polymerase (Takara Co.). After initial denaturation at 94 °C for five minutes, each thermal cycling was as follows: denaturation at 94 °C for one minute, annealing at 53 °C for one minute, and elongation at 72 °C for one minute. At the end of 30 cycles, the final extension step was at 72 °C for 15 min. Products of four parallel PCRs were combined and electrophoretically separated by 1% agarose. Finally, plant samples were determined to be successfully surface disinfected if no bacterium was identified via cultivation and PCR. These plants were used for the subsequent analyses.

DNA extraction and amplification of the bacterial 16S rRNA genes

About 2 g of surface-disinfected *G. montana* was frozen with liquid nitrogen and ground to a fine powder in a sterilized and precooled mortar. Next, the cetyltrimethylammonium bromide (CTAB) procedure was used to extract total DNA as previously described (Xie *et al.*, 1999). The DNA was resuspended in 150 μL sterile Milli-Q water. The primer pair 799f and 1492r was selected to amplify the 16S rDNA of the endophytic bacteria. The PCR reaction mixture and programs are the same as described above in the section of surface disinfection. We excised the approximately 730 bp band from a 1% agarose gel, following electrophoresis of the DNA, and purified the DNA using the Gel Extraction Kit (Omega Co.), as described by the manufacturer.

Construction of the 16S rDNA clone library

The purified 730 bp PCR products were ligated into the pMD18-T vector (Takara Co.). *Escherichia coli* Top10 competent cells (Tiangen Co.) were transformed with the ligation products and spread onto Luria-Bertani agar plates with ampicillin (100 mg L^{-1}) for standard blue and white screening (Sambrook *et al.*, 1989). Randomly selected colonies were screened directly for inserts by performing colony PCR with primers RV-M (5'-GAGCGGATAACAATTTCACACAGG-3') and M13-47 (5'-CGCCAGGGTTTTCCCAGTCACGAC-3') for the vector (Takara Co.). Two hundred fifty clones containing inserts of the correct size were sequenced using an ABI PRISM 3730 automatic sequencer (Shanghai Sangon Co., Ltd).

Phylogenetic analysis

After being trimmed by cutting the vector sequences using the Editseq program in the DNAStar package (Burland, 2000) and removing all the bad sequences as determined by the chimera sequence detection software Mallard 1.02 (www.cardiff.ac.uk/biosi/research/biosoft), all other manually verified nucleotide sequences were submitted to the NCBI GenBank database. Clones of 16S rRNA gene sequences showing 97% similarity or higher were considered to belong to the same phylotype by sequencher 4.8 (Gene Codes, Ann Arbor, MI) and assigned to an Operational Taxonomic Unit (OTU). Sequences of all phylotypes were compared to the NCBI database using BlastN or aligned by the identify analysis of EzTaxon-e *(*Kim *et al.*, 2012). Clones with a 16S rDNA sequence similarity larger than 97% were assigned to the same species; those with > 95% identity were assigned to the same genus; those with < 95% were determined to be uncultured bacterial species. Next, those sequences assigned to uncultured bacteria were aligned using Clustal W (Thompson *et al.*, 1994), and tree constructions were done with the MEGA 5 program package (Tamura *et al.*, 2011) using the neighbor-joining method (Saitou and Nei, 1987) to infer their classification. Bootstrap analysis was performed with 1,000 replicates.

Estimation of the size of the clone library

To estimate the representation of the library, the clone coverage was calculated with the following equation based on the sequencing results: $C = (1-n1/N) \times 100\%$, where $n1$ represents the number of phylotypes occurring only once and N is the number of clones being examined. Diversity of the clone library was investigated using rarefaction analysis. Rarefaction curve was calculated using the Ecosim 7.0 software (Gotelli and Entsminger, 2004).

Isolation of culturable endophytes and determination of CFU

To isolate the endophytes from the plants, 1 mL of sterile 0.85% NaCl was added to 0.5 g (fresh weight) of surface disinfected *G. montana* and samples were homogenized in a small sterile mortar. The resultant mixture was serially diluted with sterile 0.85% NaCl and plated onto R2A and TSA media (Difco, Detroit, MI). Plates were incubated for 3 days at 28 °C, after which Colony-Forming Units (CFU) were counted to calculate the average number of colonies per gram of moss. Isolates obtained by plating were purified and stored at -70 °C in sterile broth containing 40% glycerol.

ARDRA analysis and identification of the isolates by sequencing

1 uL of the bacterial suspension derived from each isolate was used to amplify the 16S rDNA fragments using the primers 27f and 1492r. The PCR reaction mixture and programs are the same as described above in the section on surface disinfection. The approximately 1490 bp band was excised from a 0.8% agarose gel, and purified using the Gel Extraction Kit (Omega Co.) as described by the manufacturer. Next, the purified products were enzymatically digested with *Hae* III and *Hha* I at 37 °C for 4 h, respectively. According to their electrophoresis pattern on a 1.0% agarose gel, these isolates were classified into different OTUs. Finally, the PCR products of isolates with different OTUs were sequenced using an ABI PRISM 3730 automatic sequencer (Shanghai Sangon Co., Ltd). After trimming the low quality nucleotides, the sequence similarities were calculated using the EzTaxon-e (Kim *et al.*, 2012).

Results

16S rDNA library analysis of endophytic bacterial community

Bacterial 16S rDNA fragments were amplified from total DNA that was extracted from surface disinfected *G. montana*, using the primers 799f and 1492r. The amplified DNA displayed only one distinct and one weak band, of approximately 730 bp and 1000 bp, respectively. The sequencing result showed that the 730 bp band represented the bacterial 16S rRNA fragment, while the 1000 bp fragment was mainly derived from the mitochondria of the mosses. Thus, the purified 730 bp PCR products were used to construct a 16S rDNA clone library for the endophytic bacteria.

Of 250 clones, two-hundred and twelve individual sequences were verified. They were determined as 90 phylotypes by sequencher 4.8 and the sequences were deposited in GenBank (Accession No.: JX042330-JX042419). Of them, 48 phylotypes occurring only once, and the calculated coverage of the clone library was 77.4%.The rarefaction curve also showed that the clones detected could reflect the main information of endophytes (Figure 1).

Sequence alignment revealed that 196 individual sequences exhibited > 95% similarity with those of cultivable bacteria. Of these, 90 clones (45.9%) were affiliated with Proteobacteria, 54 clones (27.6%) with Firmicutes, 29

(14.8%) with Actinobacteria, and 23 (11.7%) with *Cytophaga/Flavobacterium/Bacteroides* (CFB) group. Details of all alignments in the clone library are listed in Table 1.

The sequences attributed to Proteobacteria, which includes alpha, beta and gamma classes, made up the largest fraction of the clone library. Of the 90 clones affiliated with Proteobacteria, 67 clones (or 74.4%) exhibited high similarity to Gammaproteobacteria. The proportion of clones that grouped with the alpha and beta classes was 20% and 5.6%, respectively. However, there were no sequences with > 95% similarity to genera in the delta or epsilon class. The 67 clones of Gammaproteobacteria were related to four orders of bacteria, including Pseudomonadales (34 clones), Enterobacteriales (22 clones), Aeromonadales (10 clones) and Xanthomonadales (1 clone). Of these, the dominant genera include: *Acinetobacter*, *Aeromonas*, *Citrobacter, Enterobacter*, *Leclercia*, *Pseudomonas* and *Psychrobacter*; the dominant species were *Acinetobacter johnsonii*, *Acinetobacter junii*, *Leclercia adecarboxylata*, *Aeromonas punctata* and *Enterobacter cancerogenus* (Table 1). Alphaproteobacteria was the second-most abundant subgroup of Proteobacteria in our survey. The 18 clones in this subgroup represented bacteria in four orders (Rhizobiales, Sphingomonadales, Rhodobacterales and Caulobacterales) (Table 1). The dominant genera were *Brevundimonas*, *Microvirga*, *Rhizobium* and *Sphingomonas*. Of the 5 clones affiliated with Betaproteobacteria, four belonged to bacterial species in Burkholderiales and only one was grouped into Methylophilales. All of them were assigned to different genera, including *Bordetella*, *Comamonas*, *Methylophilus*, *Ramlibacter* and *Variovorax* (Table 1).

Among the non-Proteobacteria, 54, 29 and 23 clones exhibited high similarity to bacterial species in the phyla Firmicutes, Actinobacteria and CFB respectively (Table 1). In Firmicutes, 43 clones were closely related to bacteria in Bacillales, 9 clones to Clostridiales and only 2 to Lactobacillales. The dominant genera included *Paenisporosarcina*, *Planococcus*, *Planomicrobium*, and the most abundant species were *Paenisporosarcina macmurdoensis* and *Planococcus rifietoensis*. Of the 29 clones grouped into Actinomycetales of phylum Actinobacteria, twelve clones were grouped with the *Arthrobacter* genus, while the others grouped with many other genera including *Aeromicrobium* and *Ornithinicoccus* (Table 1). *Arthrobacter sulfonivorans* was the most common species. In the 23 clones belonging to the CFB phylum, bacteria occurred in four orders, the Sphingobacteriales, Cytophagales, Bacteroidales and Flavobacteriales. The dominant genera were *Adhaeribacter* and *Segetibacter*, and *Segetibacter koreensis* was the most common species.

Finally, the 16S rDNA sequence of 16 clones, showed < 95% similarity to the previously cultivated bacteria. The phylogenetic analysis showed that these clones exhibited a close relationship with Actinobacteria (4 clones), Alphaproteobacteria (3 clones), Acidobacteria (3 clones), Bacteroidetes (2 clones), Betaproteobacteria (1 clone) and Firmicutes (3 clones) (Figure 2).

Endophytic bacteria communities detected by cultivation method

The isolation result showed that the number of colony-forming units (CFU) as determined for samples grown on R2A medium was higher than the number of CFUs grown on TSA medium. The counts (expressed as g^{-1} fresh weight) were $2.0*10^5$ and $3.3*10^4$ on R2A and TSA medium, respectively. Totally 49 isolates were sequenced on the basis of 16S rDNA fragments, the ARDRA analysis resulted in the delimitation of 14 OTUs. Based on their 16S rDNA sequences (Genbank no. JX042420 - JX042433), they were assigned to 8 genera in three phyla (Proteobacteria, Actinobacteria and Firmicutes). The strains that were successfully cultivated included some genera in the Proteobacteria (*Burkholderia*, *Massilia*, *Pseudomonas*, *Spingomonas*, *Yersinia*), and some genera in Firmicutes and Actinobacteria such as *Curtobacterium*, *Brevibacterium* and *Streptomyces*. The most abundant species were *Curtobacterium flaccumfaciens*, *Massilia brevitalea*, *Pseudomonas azotoformans* and *Pseudomonas libanensis* (Table 2).

Compared the above bacterial communities with those discovered by 16S rDNA library technique, the cultivated species only involved in three phyla (Firmicutes, Proteobacteria and Actinobacteria) and no bacteria in group CFB was cultivated. The species and genera discovered by cultivation were much less than those detected by molecular method. In addition, some of genera cultivated also could not be found by molecular method, like *Curtobacterium, Massilia, Burkholderia* and *Yersinia.*

Discussion

In this study, we provide a thorough description of the endophytic bacterial community of *G. montana*, using a combined approach of molecular methods and cultivation-dependent techniques. *G. montana* individuals were sampled from stone surfaces poor in nutrient availability and subject to strong stresses, such as a wide range of temperatures and extreme drought conditions. As far as we know, ours is the first description to date of the endophytic community of a xerophilous moss species in the Grimmiaceae.

Bacterial species detected by 16S rDNA library technique belong to 4 phyla and 54 genera, with a high proportion of Gammaproteobacteia, Firmicutes and Actinobacteria. Isolates from R2A and TSA media also discovered species in these groups, no bacteria in phylum CFB was cultivated. Although some of the genera discovered by these two methods were inconsistant, it reflected that using the combination of 16S rDNA library and cultivated method would be helpful to discover the bacterial in-

formation completely. Both of them proved that many different species coexisted in this small host (G. montana).

Compared to published accounts of bacterial communities associated with other moss species growing in peat bog, such as *Sphagnum*, our study revealed the different endophytes inhabiting the tissue of *G. montana*. In previous studies, *Serratia* and *Pseudomonas* of the Gammaproteobacteria, *Burkholderia* of the beta subgroup, *Methylocella* and *Methylocapsa* of the alpha subgroup (Raghoebarsing *et al.*, 2005) and *Staphylococcus* of the Firmicutes (Opelt *et al.*, 2007) were reported to be associated with *Sphagnum* species. In this survey, of the Gammaproteobacteria subgroup, *Acinetobacter*, *Leclercia* and *Aeromonas* were the dominant genera. *Rhizobium* of the Alphaproteobacteria, *Massilia*, *Burkholderia* and five of other genera of betaproteobacteria were also detected. In addition, there were also a high proportion of Gram positive bacteria detected in our library. Of them, clones assigned to Firmicutes comprised 25.5% of the total. *Planococcus, Paenisporosarcina, Planomicrobium* and *Bacillus* were the dominant genera; while *Arthrobacter* and *Curtobacterium* of Actinobacteria were also abundant. The inconsistent endophytic bacterial community in *G. montana* and *Sphagnum* species proved that plant species and niches could cooperatively shape the structure of endophytic bacterial communities (Berg and Smalla, 2009).

Analyzing the function of those bacteria dominanted in *G. montana* would be helpful to understand the interactions between endophytes and hosts. Of gammaproteobacteria class, the dominant species *Acinetobacter johnsonii* has been reported to produce alkaline and low-temperature lipase (Wang *et al.*, 2011a); *Acinetobacter junii* was considered to be a kind of cellulolytic bacterium that can produce xylanase, cellulose and pectinase (Lo *et al.*, 2010; Zhai *et al.*, 2010) and also could remove (via accumulation) phosphate from synthetic wastewater (Hrenovic *et al.*, 2010); *Leclercia adecarboxylata* could degrade two and three benzene-ring polycyclic aromatic hydrocarbon compounds (Sarma *et al.*, 2004; Sarma *et al.*, 2010); Aeromonas veronii and *Aeromonas punctata* subsp. *caviae*, could produce enzymes such as the amino acid racemase, and xylanase (Cao *et al.*, 2007; Cruz *et al.*, 2008; Silver *et al.*, 2011). As with the *Sphagnum* bacterial communities, *Pseudomonas* was also the dominant genus in our study. The isolated species *Pseudomonas azotoformans* (Komeda *et al.*, 2004; Nie *et al.*, 2011) could degrade Cyhalofop-butyl, while *Pseudomonas libanensis* could produce the biosurfactant viscosin (Dabboussi *et al.*, 1999; Saini *et al.*, 2008). *Rhizobium pusense* of the Alphaproteobacteria was first isolated from the rhizosphere of chickpea plants and considered to be a non-symbiotic *rhizobium*. In our survey utilizing a 16S rDNA library, five clones of *Rhizobium pusense* were detected, indicating that this species could be in symbiosis with *G. montana*.

Of bacteria assigned to Firmicutes, *Planococcus rifietensis* and *Paenisporosarcina macmurdoensis* were the dominant species, which have ever been previously isolated from algal or cyanobacterial mats in sulfurous springs (Reddy *et al.*, 2003; Romano *et al.*, 2003). Four *Planomicrobium* species were also found, which have been previously isolated from coastal sediments (Dai *et al.*, 2005), seafood jeotgal (Yoon *et al.*, 2001) and glaciers (Zhang *et al.*, 2009a); they were considered as the cold tolerant bacteria (Yang *et al.*, 2011; Zhang *et al.*, 2009a). In addition, *Bacillus simplex* was isolated by cultivation, which was ever provided to have strong antioxidant activity (Wang *et al.*, 2011b). Among the Actinobacteria, *Arthrobacter sulfonivorans* could produce membrane-associated dimethylsulfone- and dimethylsulfoxide-reductases (Borodina *et al.*, 2002); *Arthrobacter agilis* could release N,N-dimethyl-hexadecanamine (dimethylhexadecylamine) to directly affect plant morphogenesis (Fong *et al.*, 2001; Velazquez-Becerra *et al.*, 2011) and could contribute to membrane stabilization in response to thermal and salt stress by increasing carotenoid accumulation (Fong *et al.*, 2001); *Curtobacterium* was a dominant genus discovered in the cultures, and *Curtobacterium flaccumfaciens*, as the most dominant species in this group, also was known to reduce symptoms caused by *Xylella fastidiosa* in *Catharanthus roseus* (Lacava *et al.*, 2007); the

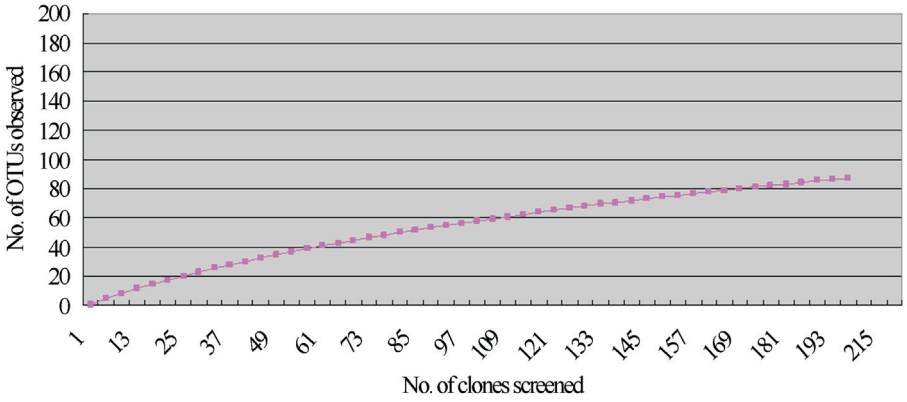

Figure 1 - Rarefaction curve for the endophytic bacterial 16S rDNA clone library of *Grimmia montana*.

cultivable *Streptomyces griseoplanus* could produce anticapsin and Erythromycin-a, and might probably help to resist pathogens in the host (Boeck *et al.*, 1971; Thompson *et al.*, 1971).

The dominant species *Segetibacter koreensis* from CFB phylum was first isolated from ginseng fields in South Korea (An *et al.*, 2007), while *Adhaeribacter tereus* and *Adhaeribacter aquaticus* were ever isolated from soil (Zhang *et al.*, 2009b) and water biofilms (Rickard *et al.*, 2005), respectively. This is the first time that these species have been found as endophytes, and their possible functions remain unclear.

In conclusion, the most important findings of this study were: (1) a high endophytic bacterial diversity and complex community structure were found associated with *G. montana,* using a combination of molecular and cultivation techniques; (2) community structure differed from that of endophytic communities of *Sphagnum* mosses, especially in the abundance of Actinobacteria and Firmicutes (higher in *G. Montana*); and (3) Some bacterial species found endophytically in *G. montana* are known to have possible beneficial effects on plants, but whether this is the case in G. Montana is not proven. Thus, in order to improve our understanding of the concrete mechanisms through which endophytic bacteria (such as those of *G. montana*) adapt to extreme environments and discover new bacterial resources, further work needs to be done in the future.

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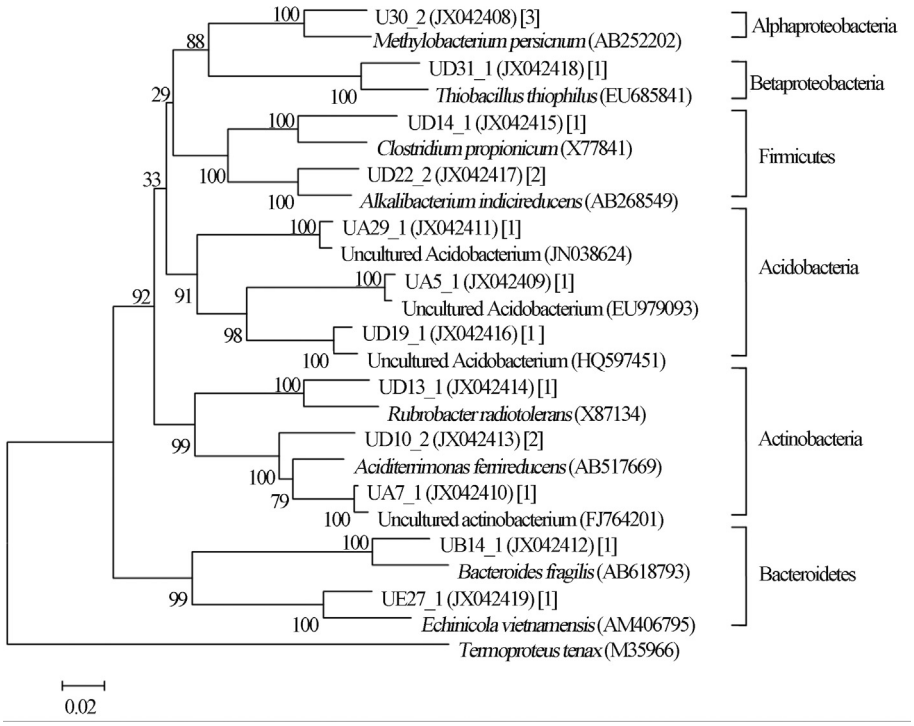

Figure 2 - 16S rDNA–based dendrogram showing the phylogenetic relationships of uncultured endophytic bacterial clones from *Grimmia montana*. Phylogeny was inferred using a neighbor-joining analysis and trees were generated using MEGA5 software. Numbers in parentheses represent the sequence accession numbers in GenBank. Numbers in square brackets indicate the number of clones out of the total clones. Numbers at branch points indicate bootstrap values. The scale bar represents a 2% estimated difference in nucleotide sequence.

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Table 1 - Identity of the clones based on 16S rDNA sequence similarity.

Group	Number of clones	The closest match	Accession No.	Sequence similarity%
Gammaproteobacteria	67			
	17	*Acinetobacter johnsonii* DSM 6963(T)	X81663	100
	3	*Acinetobacter guillouiae* ATCC 11171(T)	X81659	99
	5	*Acinetobacter junii* LMG 998(T)	AM410704	100
	11	*Leclercia adecarboxylata* GTC 1267(T)	AB273740	100
	5	*Aeromonas punctata* subsp. *caviae* ATCC 15468(T)	X74674	100
	5	*Aeromonas veronii* ATCC 35624(T)	X60414	100
	5	*Enterobacter cancerogenus* LMG 2693(T)	Z96078	99
	4	*Pseudomonas balearica* SP1402(T)	U26418	100
	1	*Pseudomonas knackmussii* B13(T)	AF039489	100
	3	*Psychrobacter pulmonis* CECT 5989(T)	AJ437696	100
	4	*Citrobacter murliniae* CDC 2970-59(T)	AF025369	100
	2	*Pectobacterium wasabiae* ATCC 43316(T)	U80199	97
	1	*Arenimonas composti* TR7-09(T)	AM229324	97
	1	*Enhydrobacter aerosaccus* LMG 21877(T)	AJ550856	99
Alphaproteobacteria	18			
	5	*Rhizobium pusense* NRCPB10(T)	FJ969841	100
	2	*Brevundimonas vesicularis* LMG 2350(T)	AJ227780	100
	2	*Microvirga aerophila* 5420S-12(T)	GQ421848	95
	2	*Microvirga subterranea* DSM 14364(T)	FR733708	97
	1	*Microvirga flocculans* TFB(T)	AB098515	98
	1	*Altererythrobacter ishigakiensis* JPCCMB0017(T)	AB363004	97
	1	*Methylobacterium brachiatum* B0021(T)	AB175649	100
	1	*Paracoccus stylophorae* KTW-16(T)	GQ281379	98
	1	*Rhodovulum euryhalinum* DSM 4868(T)	D16426	97
	1	*Sphingomonas koreensis* JSS26(T)	AF131296	98
	1	*Sphingomonas molluscorum* KMM 3882(T)	AB248285	97
Betaproteobacteria	5			
	1	Bordetella avium 197N	AM167904	99
	1	*Comamonas terrigena* LMG 1253(T)	AJ430342	100
	1	*Methylophilus flavus* Ship(T)	FJ872108	100
	1	*Ramlibacter henchirensis* TMB834(T)	AF439400	97
	1	*Variovorax dokdonensis* DS-43(T)	DQ178978	99
Firmicutes	54			
	12	*Planococcus rifietensis* M8(T)	AJ493659	100
	2	*Planococcus donghaensis* JH 1(T)	EF079063	97
	1	*Planococcus citreus* NCIMB 1493(T)	X62172	99
	1	*Planococcus maritimus* TF-9(T)	AF500007	100
	13	*Paenisporosarcina macmurdoensis* CMS 21w(T)	AJ514408	100
	3	*Planomicrobium koreense* JG07(T)	AF144750	100
	2	*Planomicrobium glaciei* 423(T)	EU036220	100
	3	*Planomicrobium chinense* DX3-12(T)	AJ697862	100
	1	*Planomicrobium okeanokoites* IFO 12536(T)	D55729	99
	2	*Anaerotruncus colihominis* DSM 17241(T)	ABGD02000032	95
	2	*Bacillus vallismortis* DSM 11031(T)	AB021198	100
	2	*Pseudoflavonifractor capillosus* ATCC 29799(T)	AAXG02000048	98

Group	Number of clones	The closest match	Accession No.	Sequence similarity%
	2	*Robinsoniella peoriensis* PPC31(T)	AF445285	96
	2	*Staphylococcus hominis* subsp. *hominis* DSM 20328(T)	X66101	100
	1	*Alkalibacterium kapii* T22-1-2(T)	AB294171	98
	1	*Atopostipes suicloacalis* PPC79(T)	AF445248	95
	1	*Finegoldia magna* CCUG 17636(T)	AF542227	100
	1	*Paenibacillus agaridevorans* DSM 1355(T)	AJ345023	98
	2	*Roseburia intestinalis* L1-82(T)	AJ312385	95
Actinobacteria	29			
	5	*Arthrobacter sulfonivorans* ALL(T)	AF235091	99
	3	*Arthrobacter agilis* DSM 20550(T)	X80748	100
	2	*Arthrobacter bergerei* CIP 108036(T)	AJ609630	100
	3	*Arthrobacter sulfureus* DSM 20167(T)	X83409	100
	3	*Ornithinicoccus hortensis* KHI 0125(T)	Y17869	98
	2	*Aeromicrobium erythreum* NRRL B-3381(T)	AF005021	99
	2	*Corynebacterium lipophiloflavum* DSM 44291(T)	ACHJ01000075	100
	1	*Agrococcus jenensis* DSM 9580(T)	X92492	100
	1	*Cellulomonas aerilata* 5420S-23(T)	EU560979	100
	1	*Geodermatophilus obscurus* DSM 43160(T)	CP001867	99
	1	*Microlunatus panaciterrae* Gsoil 954(T)	AB271051	97
	1	*Nocardioides islandensis* MSL 26(T)	EF466123	98
	1	*Sporichthya brevicatena* IFO 16195(T)	AB006164	95
	1	*Streptomyces resistomycificus* NBRC 12814(T)	AB184166	100
	1	*Tessaracoccus profundi* CB31(T)	FJ228690	98
	1	*Yonghaparkia alkaliphila* KSL-113(T)	DQ256087	100
Cytophaga/ Flavobacterium/ Bacteroides	23			
	6	*Segetibacter koreensis* Gsoil 664(T)	AB267478	98
	3	*Segetibacter aerophilus* 6424S-61(T)	GQ421847	97
	2	*Adhaeribacter terreus* DNG6(T)	EU682684	99
	1	*Adhaeribacter aquaticus* MBRG1.5(T)	AJ626894	97
	1	*Adhaeribacter terreus* DNG6(T)	EU682684	95
	2	*Bacteroides nordii* WAL 11050(T)	AY608697	95
	2	*Dysgonomonas mossii* DSM 22836(T)	ADLW01000023	95
	1	*Aequorivita sublithincola* 9-3(T)	AF170749	97
	1	*Cloacibacterium normanense* CCUG 46293(T)	AJ575430	99
	1	*Flavobacterium swingsii* WB 2.3-68(T)	AM934651	96
	1	*Ohtaekwangia koreensis* 3B-2(T)	GU117702	95
	1	*Parasegetibacter luojiensis* RHYL-37(T)	EU877263	97
	1	*Rhodocytophaga aerolata* 5416T-29(T)	EU004198	98
Uncultured bacteria	16			
	3	Uncultured bacterium	EU289421	99
	2	Uncultured bacterium	JF429066	98
	2	Uncultured actinobacterium	EF016801	98
	1	Uncultured actinobacterium	FJ764201	98
	1	Uncultured Acidobacteria bacterium	EU979093	98
	1	Uncultured Acidobacteria bacterium	HQ597451	98
	1	Uncultured Acidobacteria bacterium	JN038624	98
	1	Uncultured bacterium	EF445161	92

Group	Number of clones	The closest match	Accession No.	Sequence similarity%
	1	Uncultured bacterium	FJ479325	99
	1	Uncultured bacterium	FJ534972	94
	1	Uncultured bacterium	HQ910257	98
	1	Uncultured bacterium	FJ444700	99

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Table 2 - The cultivable endophytic bacteria isolated from *Grimmia montana.*

Group	No. of isolates	The closest match	Accession No.	Sequence similarity %
Gammaproteobacteria	21			
	11	*Pseudomonas azotoformans* IAM1603(T)	D84009	99.7
	7	*Pseudomonas libanensis* CIP 105460(T)	AF057645	99.5
	1	*Pseudomonas graminis* DSM 11363(T)	Y11150	99.9
	1	*Pseudomonas koreensis* Ps9-14 (T)	AF468452	99.9
	1	*Yersinia intermedia* ATCC 29909(T)	AF366380	99.4
Alphaproteobacteria	5			
	1	*Sphingomonas aquatilis* JSS7(T)	AF131295	98.8
	2	*Sphingomonas azotifigens* NBRC 15497(T)	AB217471	99.9
	2	*Sphingomonas melonis* DAPP-PG 224(T)	AB055863	98.7
Betaproteobacteria	12			
	1	Burkholderia glathei ATCC 29195(T)	Y17052	97.1
	11	*Massilia brevitalea* byr23-80(T)	EF546777	97.9
Actinobacteria	9			
	6	*Curtobacterium flaccumfaciens* LMG 3645(T)	AJ312209	100
	2	*Curtobacterium herbarum* P 420/07(T)	AJ310413	99.3
	1	*Streptomyces griseoplanus* AS 4.1868(T)	AY999894	99.9
Firmicutes	2			
	2	*Bacillus simplex* NBRC 15720 (T)	AB363738	99.9

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