Members of the phylum Acidobacteria are dominant and metabolically active in rhizosphere soil

Sang-Hoon Lee1, Jong-Ok Ka2 & Jae-Chang Cho1

1Department of Environmental Sciences, Hankuk University of Foreign Studies, Yong-In, Korea; 2Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

Abstract

A culture-independent survey was performed to search for 16S rRNA gene sequences representing dominant and metabolically active bacteria in rhizosphere soil. PCR- and reverse transcription-PCR-derived clone libraries were constructed from DNA and RNA directly extracted from the soil sample. Acidobacteria-related sequences occupied an unusually large proportion (>50%) of both rDNA- and rRNA-derived clone libraries. This study suggested that the bacteria belonging to the phylum Acidobacteria might be numerically dominant as well as metabolically active in the soil sample, implying that the phylum Acidobacteria might be highly involved in the biogeochemical cycles of the rhizosphere soil.

Introduction

Bacteria belonging to the phylum Acidobacteria have been observed in a wide variety of environments. They have been identified using molecular surveys based on 16S rRNA gene sequences in community DNAs, which were directly extracted from the environments, typically via PCR, cloning, and sequencing (Ludwig et al., 1997; Hugenholtz et al., 1998; Barns et al., 1999; Horn et al., 2003; Zimmermann et al., 2005; Janssen, 2006; Penn et al., 2006). To date, more than 3000 sequences represent this phylum in public databases and almost all Acidobacteria-related sequences have been recovered from uncultivated microorganisms in soil samples (Cole et al., 2005; Janssen, 2006). Considering their phylogenetic diversity and ecological distribution pattern, the phylum Acidobacteria is a metabolically and genetically diverse group compared with the well-understood phylum Proteobacteria (Hugenholtz et al., 1998; Barns et al., 1999).

Despite the ubiquity and abundance of the bacteria belonging to the phylum Acidobacteria, our knowledge of this phylum is limited because of the relatively few species that have been described. The phylum Acidobacteria contains three species (Acidobacterium capsulatum, Geothrix fermentans, and Holophaga foetida) described in ‘Berger's Manual of Systematic Bacteriology’ (Garrity et al., 2004) and four recently recognized species (Edaphobacter aggregans, Edaphobacter modestus, Chloracidobacterium thermophilum, and Terriglobus roseus) (Bryant et al., 2007; Eichorst et al., 2007; Koch et al., 2008). Although many laboratories are constantly trying to obtain pure cultures and to elucidate the ecological niche of the phylum Acidobacteria, only 99 isolates have been reported (Janssen et al., 2002; Sait et al., 2002, 2006; Joseph et al., 2003; Stevenson et al., 2004; Davis et al., 2005) and the ecological role of this phylum remains unknown.

In this study, a culture-independent survey was performed to search for 16S rRNA gene sequences representing dominant and metabolically active bacteria in a soil sample from the rhizosphere of a chestnut tree. The sampling site was located in Gyeonggi, Korea, in an area rife with this plant species, as chestnuts are the main agricultural product of this area. We analyzed 16S rDNA- and rRNA-derived clone libraries constructed from DNA and RNA directly extracted from the rhizosphere soil. Because DNA obtained from environmental samples might originate from cells in low activity or dormancy stages (Felske et al., 1997), an RNA-derived clone library was constructed to identify metabolically active members of the microbiota in the soil sample. We observed that members of the phylum Acidobacteria were dominant in both DNA- and RNA-derived
Materials and methods

Soil sample and nucleic acid extraction

Soil samples (50 g) were taken from the rhizosphere of a chestnut tree (Castanea crenata), stored at 4 °C for transport to the laboratory (< 30 min), and immediately subjected to nucleic acid extraction upon arrival. Community DNA and total RNA were directly extracted using a PowerSoil DNA isolation kit (MoBio) and an RNA PowerSoil total RNA isolation kit (MoBio), respectively. The physicochemical properties of this soil were as follows: soil texture (USDA), sandy loam; water content, 2.79%; water-holding capacity, 25.6%; total organic carbon, 0.08%; pH, 5.5; nitrate, 12 μg g⁻¹; ammonia nitrogen, 5 μg g⁻¹; aluminum, 120 μg g⁻¹; manganese, 12 μg g⁻¹; nitrite nitrogen, 1 μg g⁻¹; and sulfate, 50 μg g⁻¹.

PCR, reverse transcription (RT)-PCR, cloning, and sequencing

16S rRNA genes were directly amplified using universal primers (27F: 5′-AGAGTTTGATCCTGTCAG-3′, 1492R: 5′-AAGAGGTTGATCAGCCGCACT-3′) designed to anneal to a conserved position in the 3′ and 5′ regions of bacterial 16S rRNA genes (Massol-Deya et al., 1995). The reaction mixture included 25 μL of RED Taq ReadyMix PCR Reaction mix with MgCl₂ (Sigma), 1 μL each of the forward and reverse primers (stock concentration, 20 μM), 200 ng of template DNA extracted from the soil sample, and sterilized distilled water to give a 50-μL final volume. The PCR thermal profile was as follows: initial denaturation at 95 °C for 5 min and 30 cycles consisting of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. The final elongation step was extended to 7 min. PCR without the reverse transcription step was performed to verify the absence of DNA.

The PCR amplicons prepared from community DNA and total RNA were purified using a QIAquick PCR purification kit (Qiagen) and cloned into PCR 2.1-TOPO cloning vector with a TOPO TA cloning kit (Invitrogen) to construct rDNA- and rRNA-derived clone libraries respectively. Clones were randomly selected from the two libraries and recombinant plasmids were purified with a Plasmid Miniprep kit (Takara). Inserts were sequenced multiple times on each strand using BigDye terminator chemistry with an automated capillary sequencer (Applied Biosystems).

Phylogenetic analyses

Cloned sequences were checked for possible chimeric origins using mallard software based on the Pintail algorithm (Ashelford et al., 2005). A few potentially suspicious sequences were excluded in subsequent analyses. Phylum-level phylogenetic positions of the cloned sequences were determined using the naïve Bayesian classifier (Wang et al., 2007). RDP’s Sequence Match (RDP database) and NCBI-blast (GenBank database) were used to find closely related sequences. Cloned sequences, along with reference sequences, were edited, aligned using CLUSTALW (Thompson et al., 1994), and subjected to phylogenetic reconstruction using mega software (Kumar et al., 2004). The evolutionary distances were calculated according to Kimura’s 2-parameter model (Kimura, 1980). Phylogenetic trees were inferred using the neighbor-joining algorithm and the tree topology was statistically evaluated by 1000 bootstrap resamplings.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences obtained in this study have been deposited in the GenBank database under accession numbers EF588327–EF588427.

Results and discussion

PCR-amplified 16S rRNA genes from community DNA, extracted directly from the rhizosphere soil, were cloned, sequenced, and phylogenetically analyzed (Fig. 1). Sixty-five percent of the 49 analyzed sequences belonged to the phylum Acidobacteria, while 22% were members of the phylum Proteobacteria (Fig. 2). In the Acidobacteria clade, three bootstrap-supported monophyletic groups, which corresponded to acidobacterial subdivisions 1, 2, and 3.
(Hugenholtz et al., 1998; Janssen, 2006), were observed, and accounted for 22.5%, 36.7%, and 6.1%, respectively, of the clones in our library. The minor groups included the phyla Clostridia (8%), Verrucomicrobia (2%), and Planctomycetes (2%). These results were in contrast to those from preliminary studies using cultures. Cultures on both oligotrophic (R2A agar, Difco) and copiotrophic (Nutrient agar, Difco) media showed that the majority (99%) of isolates belonged to the phylum Firmicutes likely due to culturing biases (data not shown).

Unlike most other soil bacterial community structures determined by 16S rRNA gene clone libraries, the phylum Acidobacteria occupied an unusually large proportion (65%) of our clone library and outnumbered the phylum Proteobacteria by approximately three to one. Although our observations should not be generalized to the soil microbial community because of the limited size of our clone library and an additional sampling of bacterial clones required to determine the full extent of bacterial community diversity, our results do nevertheless suggest that the phylum Acidobacteria in rhizosphere soil

Fig. 1. Phylogenetic relationships of PCR-amplified 16S rRNA gene sequences from the rhizosphere soil. The cloned sequences were compared with the most closely related sequences obtained from the database (RDP-II), as well as other representatives of major bacterial groups. The phylogenetic distances of each sequence were calculated using the Kimura 2-parameter model and the tree was constructed using the neighbor-joining algorithm. The numbers at the nodes indicate the bootstrap score (as a percentage) and are shown for frequencies at or above the threshold of 50%. The scale bar represents the expected number of changes per nucleotide position.
Acidobacteria might be the predominant bacterial group in our rhizosphere soil sample.

In a comprehensive review of the dominant soil bacterial taxa in 16S rRNA gene libraries (Janssen, 2006), members of the phylum Proteobacteria (black bar) and phylum Acidobacteria (gray bar) made up an average of 39% (range, 10–77%) of libraries constructed from soil bacterial communities, and the members of the phylum Acidobacteria (LaPara et al., 2000; Layton et al., 2000; Sievert et al., 2000; Smit et al., 2001; Paster et al., 2002; Martiny et al., 2003; Polymenakou et al., 2005; Penn et al., 2006). To our knowledge, there are only two other reports that show that the phylum Acidobacteria contributes substantially (> 50%) to the total bacterial community. In the study conducted by Kuske et al. (1997), the phylum Acidobacteria was the most dominant bacterial group in 16S rRNA gene clone libraries, which were constructed from soil samples taken from the Sunset Crater National Monument (53.6%) and Coconino National Forest (57.1%) in Arizona, United States. Later, Dunbar et al. (1999) observed that Acidobacteria-related sequences were most abundant in clone libraries constructed from soil samples collected from the same geographic location. Based on the distribution of 16S rRNA gene sequences among the bacterial phyla found in the literature, Smit et al. (2001) suggested that the ratio between the number of Proteobacteria and Acidobacteria might be determined by the trophic level of the soil. The ratio of Proteobacteria to Acidobacteria was < 0.34 for oligotrophic soil and > 0.46 for copiotrophic soil, indicating that oligotrophic environments have a selective effect for Acidobacteria. They considered the two Arizona soils (Kuske et al., 1997; Dunbar et al., 1999) to be oligotrophic (ratio, 0.16). Based on their hypothesis, the trophic level of our rhizosphere soil sample could be marginally oligotrophic (ratio, 0.34). However, Marilley et al. (1999) found that the rhizosphere, which is a relatively copiotrophic niche for bacteria, has a selective effect toward the phylum Proteobacteria and is detrimental to the phylum Acidobacteria.

To determine the metabolically active members of bacterial community in our soil sample, we extracted total RNA from the soil sample and constructed a crDNA (complementary ribosomal RNA gene) library. Because the ribosome per cell ratio is approximately proportional to the growth rate of bacteria, and a higher number of ribosomes suggests metabolically active cells (Wagner, 1994; Pace, 1997), such 16S crDNA libraries are assumed to better reflect metabolically active bacteria. Indeed, the rrr operon copy number, which is largely unknown for uncultured bacteria, did not affect the level of rRNA (Fogel et al., 1999).

RT-PCR amplicons were cloned, sequenced, and phylogenetically analyzed (Figs 2 and 3). Fifty-six percent of 52 analyzed clones fell into the phylum Acidobacteria, while 31% was related to the phylum Proteobacteria. Similar to the result from the clone library constructed with the community DNA, three subdivisions 1, 2, and 3 were observed in the phylum Acidobacteria, representing 17.3%, 9.6%, and 28.8%, respectively, of the total crDNA library. To compare the sequences in crDNA library and rDNA library, we used a local alignment approach using NCBI-BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), because the RT-PCR-amplified region of 16S rRNA gene was different from the PCR-amplified region of 16S rRNA gene (to avoid inhibition of reverse transcription caused by the secondary structure of the 16S rRNA gene molecules). The NCBI-BLAST showed that the closest relatives of Acidobacteria-related sequences in DNA- and RNA-derived clone libraries were identical. This suggested that the cloned 16S rRNA genes could originate from the highly similar phyotypes that dominated the DNA-derived clone library. Hence, we concluded that the phylum Acidobacteria might be numerically dominant as well as metabolically active in our rhizosphere soil. However, because we performed the nucleic acid extractions after the soil samples were transported to the laboratory, changes in population structure as well as the rRNA composition might occur during the sample transportation even at 4 °C. We considered our conclusions to be valid under the assumption that no significant changes occurred during the sample storage period.

The phylum Acidobacteria occupied a large proportion of DNA- and RNA-derived clone libraries, indicating that the
Fig. 3. Phylogenetic relationships of RT-PCR-amplified 16S rRNA gene sequences from the rhizosphere soil. The cloned sequences were compared with the most closely related sequences obtained from the database (RDP-II), as well as other representatives of major bacterial groups. The phylogenetic distances of each sequence were calculated using the Kimura 2-parameter model and the tree was constructed using the neighbor-joining algorithm. The numbers at the nodes indicate the bootstrap score (as a percentage) and are shown for frequencies at or above the threshold of 50%. The scale bar represents the expected number of changes per nucleotide position.
members of the phylum *Acidobacteria* might be highly involved in biogeochemical cycles in the soil. Although *Acidobacteria*-related sequences have been retrieved from diverse environments, this ubiquitous bacterial taxon is known as an uncultured bacterial group (at least it is difficult to culture from environmental samples), and its ecological function remains largely unknown. The development of effective cultivation methods, followed by genetic and physiological characterization of the bacteria belonging to the phylum *Acidobacteria*, as well as the metagenome analysis suggested by Quaiser et al. (2003), is a relevant and ecologically significant task that would elucidate their ecological roles.

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**References**


**Supplementary material**

The following supplementary material for this article is available online:

**Table S1.** Summary of PCR-amplified 16S rRNA gene sequences from rhizosphere soil.

**Table S2.** Summary of reverse transcription-PCR-amplified 16S rRNA sequences from rhizosphere soil.

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1574-6968.2008.01232.x (This link will take you to the article abstract.)

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