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SHORT COMMUNICATION

Phylogenetic diversity of *Acidobacteria* in a former agricultural soil

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Although *Acidobacteria* represent the most abundant bacterial phylum in many soils, knowledge of acidobacterial diversity is still rather incomplete. We, therefore, examined the diversity of 16S rRNA genes affiliated with this phylum in a former arable soil via three independent approaches: (1) screening of a fosmid metagenome library for inserts containing *Acidobacteria*-like 16S rRNA genes; (2) PCR-cloning using general bacterial primers; and (3) PCR-cloning with acidobacterial-specific primers. Bacterial-specific libraries compared rhizosphere versus bulk soil samples, revealing a higher proportion of acidobacterial sequences in bulk soil libraries ($P<0.001$). Bacterial libraries recovered the greatest diversity, and sequence examination suggested that sequence mismatches with the *Acidobacteria*-specific primers limited the coverage of the metagenome library screening and specific library approaches. Together, these results expand knowledge of the distribution and diversity of *Acidobacteria* in soil environments and highlight important technical considerations in the molecular analysis of *Acidobacteria* diversity.
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Acidobacteria are among the most dominant phyla in soil-borne microbial communities. This phylum has consistently been detected as a major proportion of soil 16S rRNA gene clone libraries (Janssen, 2006). However, despite their dominant presence, relatively little is known about their distribution, diversity and function in soils. Several phylogenetic analyses have defined eight subdivisions within the *Acidobacterium* group (Hugenholtz *et al.*, 1998; Sait *et al.*, 2002; Handelsman, 2004), although some authors have suggested the presence of eleven or more subdivisions (Zimmermann *et al.*, 2005; Barns *et al.*, 2007).

Although, *Acidobacteria* have generally been refractory to classical cultivation, recent developments in improving cultivation methods have yielded a number of new soil isolates affiliated with subdivisions 1–4 (Janssen *et al.*, 2002; Sait *et al.*, 2002, 2006; Joseph *et al.*, 2003; Stevenson *et al.*, 2004; Eichorst *et al.*, 2007). However, cultivation-independent approaches, based upon 16S rRNA gene sequence analysis, have revealed a greater diversity of *Acidobacteria* in soil, with subdivision 6 typically being most highly represented in clone libraries (Janssen, 2006).

In this study, we examined acidobacterial diversity using several samples collected from a former arable field with soil described as loamy sand (Van der Putten *et al.*, 2000) and compared the diversity recovered via three independent molecular approaches: bacterial-specific PCR-cloning, *Acidobacteria*-specific PCR-cloning and the screening of metagenome fosmid library for acidobacterial rRNA genes. We hypothesized that the diversity and relative proportions of *Acidobacteria* sequences recovered from different soil compartments (rhizosphere versus bulk soil) would differ, and that cloning approach would also influence the diversity and distribution of the sequences recovered.

Detailed descriptions of the soil samples used and the construction of the different libraries are given in Supplementary materials, and summarized in Figure 1. PCR for the construction of general bacterial libraries was performed with primers pA and 1492r (Edwards *et al.*, 1989), and phylum-specific libraries, as well as PCR-based metagenome library screening, utilized primers Acd31f and 1378r (Barns *et al.*, 1999). The acidobacterial origin of sequences was confirmed by database comparisons and phylogenetic analysis (Figure 2 and Supplementary Table S1, Figures S1 and S2).

Within general bacterial libraries, a higher proportion of *Acidobacteria* sequences was observed in

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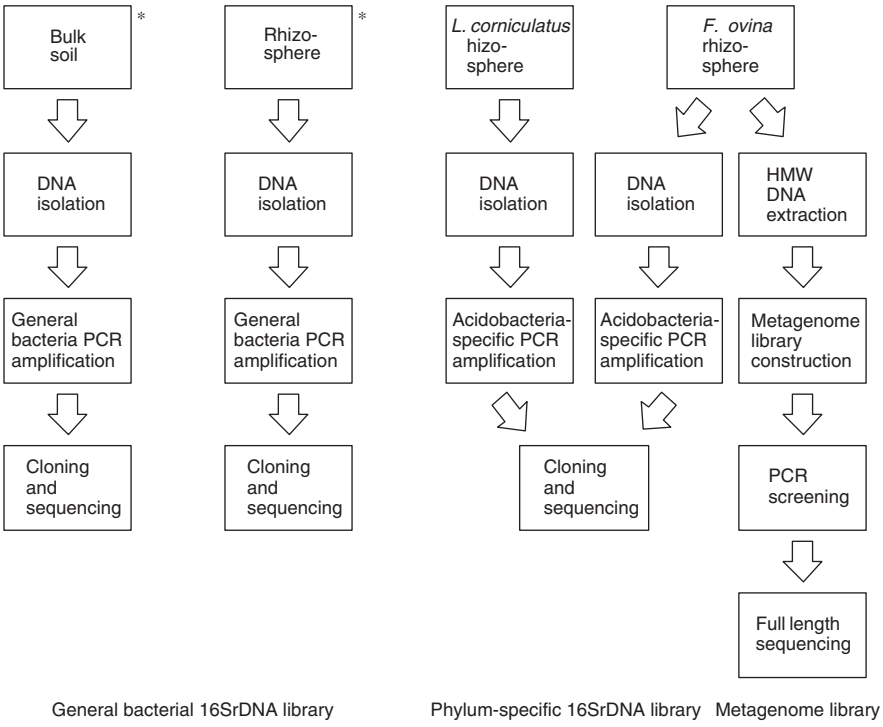

Figure 1 Summary of library construction strategies used in this study (PCR-based general bacterial and phylum-specific libraries, and PCR-independent metagenome library). Each general bacterial library consisted of four independently constructed libraries from soils collected from different field sowing treatments (see Van der Putten *et al.*, 2000; Kielak *et al.*, 2008 and Supplementary materials for further details). *Libraries constructed in parallel from samples collected from four plots with different treatments (Kielak, 2008).

bulk soil libraries (25.1 ± 14.4% of sequences across four bulk soil libraries versus 14.7 ± 7.7% of sequences across four rhizosphere libraries), in agreement with earlier studies (Marilley and Aragno, 1999; Sanguin *et al.*, 2006; Fierer *et al.*, 2007). Also, the estimated species richness (Chao1) was higher in the bulk soil (140 ± 54 versus 72 ± 34), although this difference was not significant. The preference of *Acidobacteria* for bulk soil conditions has been suggested to be a result of the oligotrophic lifestyle thought to hold for many members of this phylum (Fierer *et al.*, 2007). Higher carbon availability in the rhizosphere is hypothesized to support fast-growing microorganisms, which presumably outcompete *Acidobacteria*.

Compared with the general bacterial libraries, which recovered a total of eight different *Acidobacteria* subdivisions, the phylum-specific and metagenome libraries recovered a more limited range of *Acidobacteria* diversity (four subdivisions each), reflected in the lower Chao1 diversity estimations (R + B = 160 (± 51), Acido = 107 (± 58), and Meta = 47 (± 22)).

Phylogenetic analyses (neighbor-joining and Bayesian inference) conducted on 157 sequences from this study (B = 101, R = 30, Acido = 28, Meta = 17), aligned with 313 sequences from the Silva database (Pruesse *et al.*, 2007), generally supported the earlier-proposed classification (Hugenholtz *et al.*, 1998; Zimmermann *et al.*, 2005), although 12 clones could not be assigned within currently recognized subdivisions, and subdivision 10 appeared to be polyphyletic (Supplementary Figure 1S and 2S). Subdivisions 6, 4, 3 and 1 were generally most abundant across the different libraries (Table 1), in agreement with other sequences recovered earlier from soil habitats (Kuske *et al.*, 1997; Barns *et al.*, 1999; Janssen, 2006; Eichorst *et al.*, 2007; Hansel *et al.*, 2008). None of the approaches yielded clones affiliated with subdivisions 8, 9 or 11.

For subdivisions 3 and 6, significant differences ($P = 0.006$) between the libraries were observed, with subdivision 3 relatively overrepresented and subdivision 6 underrepresented in the general bacterial library (Table 1). Sequences belonging to subdivisions 2, 7 and 10 were found only in the general bacterial libraries, but did not represent a large proportion of these libraries. Although different cloning strategies were carried out on soil samples taken from slightly different locations in the field experiment and in different years, previous general and *Acidobacteria*-specific PCR-denaturing gradient gel electrophoresis analyses of these and other rhizosphere samples from this field, as well as amplified ribosomal DNA restriction analysis of clone libraries, have revealed highly consistent community structure of *Acidobacteria* across different field treatments, plant species and years (Kowalchuk *et al.*, 2002; Kielak *et al.*, 2008). Furthermore, soil parent material has been

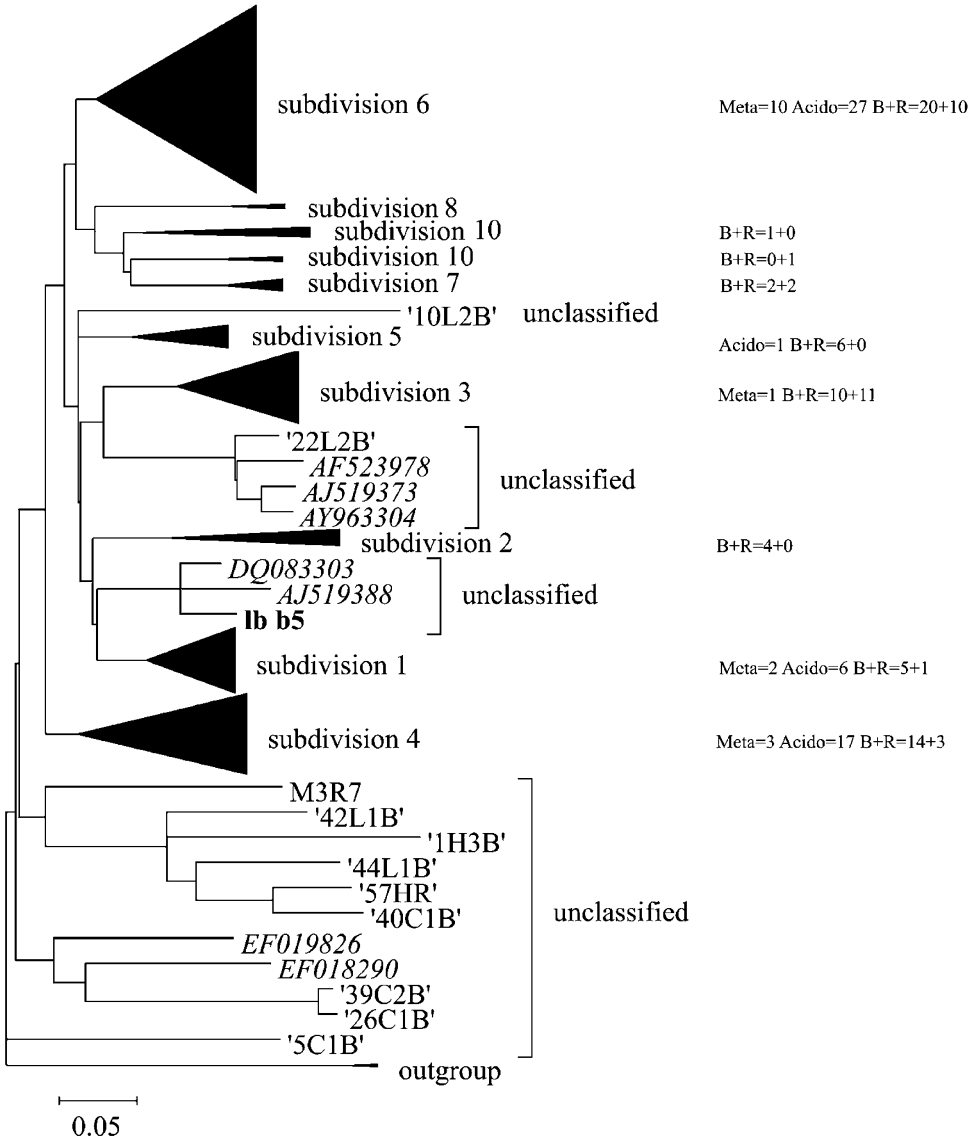

Figure 2 Simplified neighbor-joining tree of the phylum *Acidobacteria*, highlighting the sequences recovered in this study. The full tree is included in Supplementary materials (Supplementary Figure 1S). Names in italics indicate sequences retrieved from GenBank. The numbers of sequences in each subdivision recovered by the metagenome (Meta), *Acidobacteria* phylum-specific (Acido), and general bacterial (split into bulk soil (B) and rhizosphere libraries (R)) approaches are given to the right of the corresponding subdivision wedges. The depth of wedges reflects the branching depth of a particular subdivision and its opening angle represents the number of sequences.

highlighted as the main determinant of soil-borne microbial communities in arable fields (Ulrich and Becker, 2006), and the field site from which all samples originated was specifically chosen for its uniform soil characteristics. Thus, we would conclude that the differences in phylum distribution and diversity across the different cloning strategies are most probably more because of methodological differences than because of true variation in the field.

The examination of the primer binding sites within the various *Acidobacteria* subdivisions revealed several mismatches (Table 2). A particularly critical mismatch at the 3′ end of the Acd31F primer most likely affects the efficiency of primer binding for a majority of sequences from subdivisions 2 (139 out of 140), 7 (26 out of 27), 8 (29 out of 36) and 10 (83 out of 85). All acidobacterial clones in the metagenome library showed a perfect match to the Acd31f primer, suggesting that a small number of *Acidobacteria* fosmids in our library (28 800 clones) from subdivisions other than 1, 3, 4 and 6 could have gone undetected by the specific PCR screening method used.

The *Acidobacteria*-like 16S rRNA gene sequences obtained in this study add significantly to the present knowledge of the diversity within the *Acidobacteria* phylum in soil habitats. This study also highlights the need for designing new primers

Table 1 Percentage of sequences affiliated with each detected *Acidobacteria* subdivision in the metagenome (Meta), general bacterial (Bacterial; including bulk soil (B) and rhizosphere (R)), and phylum-specific (Acido) libraries

Subdivision	*Meta*	*Bacterial*			*Acido*
		B	*R*	*BR*	
1	11.8	7.0	3.3	5.9	15.8
2	0	5.6	0	4.0	0
3	5.9	14.1	36.7	20.8[a]	0[a]
4	17.6	19.7	10	16.8	10.5
5	0	8.5	0	5.9	2.6
6	58.8	28.2	33.3	29.7[a]	71.1[a]
7	0	2.8	6.7	4.0	0
10	0	1.4	3.3	2.0	0
Unclassified	5.9	12.7	6.7	10.9	0
OTU richness (Chao1)	47 ± 22	140 ± 54	72 ± 34	160 ± 50	107 ± 58

Meta, 17 clones; Bacterial, 101 clones; B, 71 clones; R, 30 clones; Acido, 38 clones.
Operational taxonomic units (OTUs) were defined at the 95% sequence identity level.
[a]Significant difference ($P<0.05$) from corresponding expected frequencies.

Table 2 Alignment of the Acd31F primer-binding site from illustrative sequences affiliated with different *Acidobacteria* subdivisions

Subgroup	*Mismatch/All*	*Sequence*							
		Acid31f	GAT	CCT	GGC	TCA	GAA	TC	
1	16/412	lbb10							
2	139/140	26L2B	-	-	-	-	-	G	CG
		38H2B	-	-	-	-	G	-	G
3	19/25	lba7	-	-	-	-	-	-	-
4	14/172	lbb4	-	-	-	-	-	-	-
5	2/24	17L2B	-	-	-	-	-	-	-
6	33/337	Lbb9	-	-	-	-	-	-	-
7	26/27	M1B24	-	-	-	-	-	-	-
8	29/36	AM180889	-	-	-	-	-	-	G
9	0/11	EU373952	-	-	-	-	-	-	G
10	85/83	AB240310	-	-	-	-	-	-	-
			-	-	-	-	-	-	G

The perfect positions of primer hybridization are shown as a line (-), letters indicate mismatch positions.

and probes not only to be more inclusive to the breadth of diversity within this phylum, but also to target specific subdivisions (Barns *et al.*, 1999, 2007; Kleinsteuber *et al.*, 2008).

Sequence accession numbers

Sequences described in this study have been deposited in The GenBank database under accession numbers FJ004643-FJ004798.

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