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MINIREVIEWS

Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes

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From near to far,
from here to there,
funny things are everywhere.
—Dr. Seuss, *One Fish Two Fish Red Fish Blue Fish*

HISTORICAL ASPECTS

In 1909, H. Joel Conn (25) expressed the hope that methods would soon be at hand by which the significance of the different bacteria present in any soil could be determined. However, by 1918 he was pointing out that the methods available to him, which relied on cultivation of bacteria on artificial media, resulted in the formation of colonies by only 1.5 to 10% of the bacterial cells in soil (26). Fifty years later, Vagn Jensen concluded a review of cultivation-based methodologies by stating his suspicion that those cells that were forming colonies were unrepresentative of the total bacterial community (59). This was confirmed when cultivation-independent methods began to be used to study soil bacteria (see below). Even so, in the absence of better methods, the pure cultures derived from the colonies that did form were extensively and successfully studied throughout the 20th century. Much of our basic knowledge of soil bacteria, as well as the discovery of many important antibiotics, came from investigations of pure cultures (2, 86, 104). Cultured isolates are still very important in developing our understanding of bacterial physiology, genetics, and ecology (85, 122).

Beginning in the 1990s, the application of molecular ecological methods, especially those based on surveys of genes after PCR amplification, has allowed cultivation-independent investigations of the microbial communities of soils to be made. The power of these methods has largely rendered obsolete the plate count approach to detecting and enumerating subsets of soil bacteria, and a range of diagnostic and quantitative methods that target functional genes, phylogenetically informative genes, or RNAs has been developed (49, 69). In particular, 16S rRNA and its gene have proven to be useful and powerful markers for the presence of bacteria in samples (36, 56, 88). The utility of these markers is facilitated by the availability of primers that allow amplification of almost the complete gene or its RNA product (66) and by the phylogenetic inferences that can be made from the resultant nucleotide sequences, permitting placement of the host organism within a phylogenetic framework even if closely related cultured organisms are lacking (36, 56, 74). Since the initial pioneering studies to survey soil bacterial communities using molecular ecological surveys (13, 68, 79, 91, 111), a number of libraries of 16S rRNA and 16S rRNA genes derived from soils have become available (Table 1).

It is important to realize that libraries of PCR-amplified 16S rRNA and 16S rRNA genes may not represent a complete or accurate picture of the bacterial community. Firstly, the species diversity is so great (28, 46, 109) that libraries of <400 cloned sequences must represent only an incomplete sampling. Even all of the currently published sequences combined would seem to constitute an incomplete census of all of the 16S rRNA genes on earth (98). In addition, there may be biases in the contributions of the various bacterial groups to libraries. The efficiencies of nucleic acid extraction may be different for different bacteria, the number of copies of 16S rRNA or 16S rRNA genes per cell varies, and there may be preferential amplification of some sequence types relative to others by PCR (36, 43, 113). Some sequences may arise from contaminating DNA and may not represent bacteria actually present in the sample being studied (108). Assigning physiologies and functions to the hosts of 16S rRNA gene sequences is complicated in many cases by the lack of characterized close relatives (e.g., see references 31, 57, 81, and 88) and by the diversity of phenotypes among close relatives in some groups (1, 95). Some, but not all, of these biases may be overcome as metagenomic data sets accumulate (71, 87, 110). In the meantime, the available libraries of 16S rRNA and 16S rRNA genes permit an initial survey of the global soil bacterial community structure.

SEQUENCE DATA SETS

Thirty-two libraries of 16S rRNA and 16S rRNA genes of members of the domain *Bacteria*, prepared from a variety of soils, were analyzed to gain an understanding of the general composition of soil bacterial communities (Table 1). Libraries or sequences from rhizosphere samples were not included in this synthesis. Libraries consisting predominantly of sequences of <300 nucleotides were also excluded, as phylogenetic assignment from very short sequences can be unreliable (36, 74), and sequences of <300 nucleotides were removed from the libraries that were included. Some published libraries were

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TABLE 1. Libraries of 16S rRNA or 16S rRNA genes prepared from RNA and DNA extracted from soils[a]

Library	Source[b]	No. of clones	No. of sequences	Reference(s)
NMS8	Canada, forest, DNA	91	67	5
NMW3	Canada, forest, DNA	90	61	5
NOS7	Canada, forest, DNA	92	73	5
NOW2	Canada, forest, DNA	93	76	5
SMS9	Canada, forest, DNA	94	83	5
SMW4	Canada, forest, DNA	92	56	5
FVM	Brazil, forest, DNA	48	48	12
FVP	Brazil, pasture, DNA	49	49	12
C0	United States, arid woodland, DNA	162	162	32, 33, 65
C1	United States, arid woodland, DNA	109	109	32, 33, 65
S0	United States, arid woodland, DNA	163	163	32, 33, 65
S1	United States, arid woodland, DNA	102	102	32, 33, 65
a13	United Kingdom, DNA*	39	38	35
HB	United States, cropping rotation, DNA	95	95	45
Tc	Switzerland, RNA + DNA*	141	141	50
Kolm	Austria, forest, DNA	45	45	51
Roth	Austria, forest, DNA	51	51	51
ST	Austria, forest, DNA	41	41	51
Sturt	Australia, arid landscape, DNA	122	122	54
WMARS00	United States, DNA*	258	258	71
WMARS97	United States, DNA*	138	138	71
Niwot	United States, alpine meadow, DNA	180	159	72
LBS	Switzerland, pasture, DNA	27	27	76
TBS	Switzerland, pasture, DNA	26	26	76
saf	United Kingdom, pasture, DNA	137	137	77
s1	United Kingdom, pasture, DNA	137	137	77
Witt	Germany, moorland, RNA + DNA	396	396	82, 83
C6	Germany, forest, DNA	31	31	92
MTP	United States, wheat, DNA	93	93	105
ABS	United States, grassland, DNA	59	59	121
DS	United States, grassland, DNA	62	62	121
EB	Australia, pasture, DNA	135	135	—[c]
Totals		3,398	3,240	

[a] All libraries were used to ascertain the genus and family affiliations of soil bacteria, but only libraries with ≥90 clones were used to determine phylum and class level abundances.
[b] *, details of vegetation not given.
[c] L. Schoenborn and P. H. Janssen, unpublished data (GenBank accession no. AY395320 to AY395454).

generated with primers that could not be expected to sample most known bacteria or were screened in such a way that the total number of clones belonging to each group could not be deduced from the published data. These were not included in this survey. Two potentially interesting libraries containing unexpectedly large numbers of sequences assignable to the genus *Escherichia* were excluded because these may not have originated from DNA from the soil being investigated (30). As a consequence of the exclusions, the final data set is not as geographically comprehensive as it might have been, but a number of different vegetation types are included (Table 1). Some libraries consist of data from multiple reports in which the sample site or the sample itself appeared to be the same. In a few cases, multiple libraries that originated from highly similar replicate samples were pooled to increase the library size. A total of 3,240 sequences from the 32 libraries were assigned to genus level groupings by the "Classifier" program of Ribosomal Database Project II (24) and then weighted for multiple clone assignments to one sequence type, and this pooled data set of 3,398 clones was treated as one global set.

The contribution of phylum level groupings to soil bacterial communities was calculated only from the 21 libraries with ≥90 clones. Smaller libraries (<90 clones) contained representatives of very few phyla. It was felt that the smaller libraries might skew the outcomes, since the contributions were normalized to compensate for library size and then analyzed further. For some of the better-characterized dominant phyla (*Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia*), the clones were also assigned to subphylum groups (class, subclass, or subdivision). Some of these subphylum groups are organizational rather than evolutionarily equivalent lineages, especially the classes of the phylum *Proteobacteria* (74), but are useful for the purposes of surveying the global data set. These assignments were based on results obtained with Classifier (17), published phylogenetic trees and tables in publications or their supporting material, BLAST (3) in GenBank databases (8), Ribosomal Database Project II databases (24), and phylogenetic analyses of sequences against references of known affiliation (e.g., see references 60, 93, and 96).

HISTORICALLY IMPORTANT SOIL BACTERIA

In his landmark book, the second edition of *Introduction to Soil Microbiology*, Martin Alexander (2) listed what were then consid-

TABLE 2. Abundance of members of well-known genera of soil bacteria in libraries of 16S rRNA and 16S rRNA genes compared with their historical significance as colony-forming soil bacteria and their contribution to soil isolates held in the ATCC

Genus	Contribution to libraries (%) at different confidence levels[a]		Range of abundance among colonies (%)[b]	Contribution to soil isolates in ATCC (%)[c]
	100%	≥80%		
Actinomadura	0	0	—[d]	1.5
Actinoplanes	0	0.06	—	1.5
Agrobacterium	0	0	0–13	—
Alcaligenes	0	0	1–8	—
Arthrobacter	0.33	0.53	3–40	1.3
Bacillus	0.18	0.62	5–45	7.6
Clostridium	0.03	0.09	—	1.6
Flavobacterium	0.35	0.38	1–7	—
Flexibacter	0	0	—	1.2
Hyphomicrobium	0.03	0.03	—	1.2
Micromonospora	0	0	0–5	2.1
Mycobacterium	0.33	0.50	—	2.6
Nocardia	0	0	3–10	—
Paenibacillus	0.12	0.18	—	1.4
Pseudomonas	1.60	1.60	2–10	6.0
Ralstonia	0	0	—	1.0
Rhodococcus	0	0	—	1.4
Streptomyces	0.06	0.06	23–30	25.2

[a] Confidence levels calculated by the program Classifier of the Ribosomal Database Project (24).
[b] Calculated from the data of Alexander (2), assuming a mean of one-third of colonies being actinomycetes (filamentous members of the subclass *Actinobacteridae*, including *Micromonospora*, *Nocardia*, and *Streptomycetes*) and the remaining two-thirds being other non-actinomycete bacteria (2).
[c] Data from Floyd et al. (41).
[d] —, no data.

ered to be important genera of soil bacteria, based on cultivation studies. He suggested that members of nine genera were significant in soils: *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Micromonospora*, *Nocardia*, *Pseudomonas*, and *Streptomyces* (Table 2). Since 1977, two things that have changed the validity of this list have occurred. First, many of the genera have undergone taxonomic revision, and some of their species have been reclassified into new or other genera. This is especially true for the genera *Flavobacterium* and *Pseudomonas* (4, 9). Second, surveying of 16S rRNA genes in soils has permitted a more direct census of soil bacteria, without the limitations inherent in cultivation-based studies. These surveys suggest that members of Alexander's nine genera, as they are currently defined, together make up only 2.5 to 3.2% of soil bacteria (Table 2). Of the nine genera, *Pseudomonas* spp. are the most abundant in soil bacterial communities, contributing 1.6% of the cloned sequences from soils (Table 2).

Recently, Floyd et al. (41) presented a breakdown of cultures of prokaryotic organisms in the American Type Culture Collection (ATCC). The 14 genera of soil bacteria with the most deposited cultures, together encompassing over half of all soil-derived isolates in the ATCC, are also not common among the clones detected in libraries (Table 2). Together, members of these genera make up only 2.7 to 3.7% of soil bacteria. This shows that the cultured part of soil bacterial diversity is not representative of the total diversity, as suggested by Jensen (59) and many others since.

TABLE 3. Assignment of cloned 16S rRNA and 16S rRNA genes affiliated with well-characterized subphylum groupings (class or subclass) to described genera

Subphylum	Proportion of all 3,398 clones (%)	Proportion of clones assignable to genera (%) at different confidence levels[a]		Total no. of described genera in group[b]
		100%	≥80%	
Actinobacteridae	4.25	1.12 (26)	2.01 (47)	158
Flavobacteria	0.50	0.35 (70)	0.38 (76)	25
Sphingobacteria	4.34	1.15 (26)	2.60 (60)	28
Bacilli	1.89	0.38 (20)	1.03 (54)	79
Clostridia	0.59	0.06 (10)	0.27 (46)	135
Alphaproteobacteria	18.55	2.10 (11)	6.38 (34)	160
Betaproteobacteria	10.87	3.31 (30)	4.82 (44)	93
Deltaproteobacteria	2.84	0.03 (1)	0.59 (21)	70
Gammaproteobacteria	7.77	2.10 (27)	2.60 (33)	194
Total	51.60	10.60	20.68	942

[a] Confidence levels calculated by the program Classifier of the Ribosomal Database Project (24). The percentage of sequences affiliated with each class that were able to be assigned to a genus is given in parentheses.
[b] Data from Garrity et al. (48).

GENUS LEVEL DIVERSITY

The Classifier algorithm (24) returns a confidence value with which a 16S rRNA gene sequence can be assigned to a taxon (genus and higher) that is represented by a set of sequences, based on the number of times, out of 100 trials, that random subsets of the query sequence match sequences assigned to that taxon. The algorithm also returns the name of the taxon to which the sequence was most often assigned in those 100 trials. Using Classifier, as many as 17% of the sequences could be assigned to a known genus with 100% confidence; this increased to 32% with a confidence level of 80% or greater. However, these outcomes were greatly influenced by sequences falling into poorly defined groups with very few described species. Sequences affiliated with these classes or phyla tended to be identified as members of the one genus or few genera in them, because there were no other genera to draw the sequences during bootstrap analysis. Nearly all of these assignments were spurious, as the sequence similarities to the few named species were <96%. Everett et al. suggest that 16S rRNA gene sequence similarities of <96% are indicative of the hosts of the genes belonging to different genera (37). When the genera *Acidobacterium* (phylum *Acidobacteria*), *Alterococcus*, *Verrucomicrobium*, *Xiphinematobacter* (phylum *Verrucomicrobia*), *Gemmatimonas* (phylum *Gemmatimonadetes*), and *Conexibacter* and *Rubrobacter* (subclass *Rubrobacteridae* of the phylum *Actinobacteria*) were removed, the number of sequences able to be assigned to known genera decreased. In their absence, only 11% of sequences could be assigned to known genera with 100% confidence and 21% at ≥80% confidence. These figures are still perhaps surprisingly high, given that soil bacterial diversity is high and our ability to culture these bacteria is generally considered to be poor (28, 46, 88, 98, 109, 122). It seems that our ability to culture representatives of the phylogenetic diversity of soil bacteria, at least as judged at the genus level, is better than the 1% often quoted, even if culturability as a function of cell numbers is low.

The majority (79 to 89%) of 16S rRNA gene sequences are from bacteria that are not affiliated with known genera. Some of

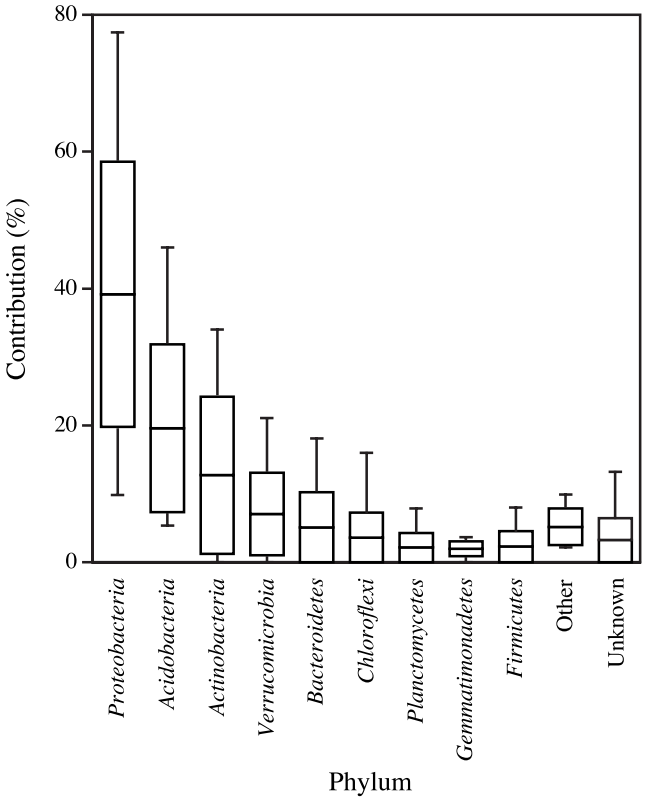

FIG. 1. Contributions of 16S rRNA and 16S rRNA genes from members of different phyla in libraries prepared from soil bacterial communities (2,920 clones in 21 libraries). The horizontal line in the middle of each block indicates the mean, the block represents 1 standard deviation on either side of the mean, and the vertical lines extending above and below each block indicate the minimum and maximum contributions of each phylum.

these are associated with well-studied lineages of bacteria, such as *Actinobacteridae*, *Flavobacteria*, *Sphingobacteria*, *Bacilli*, *Clostridia*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*. In some of these groups, the number of bacteria affiliated with known genera is high. For example, up to 60% of sequences from members of the class *Sphingobacteria* and up to 76% of sequences from members of *Flavobacteria* may come from described and named genera (Table 3). Among sequences assigned to *Actinobacteridae* this is lower, with less than half belonging to described genera, while in the *Deltaproteobacteria*, the number of sequences assignable to known genera is even lower (Table 3). Since members of the latter two groups have been the source of many chemically novel bioactive compounds (104), this indicates considerable scope for more discovery. In some groups well represented by cultured isolates, such as in the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, less than half of all sequences could be assigned to these described and named genera (Table 3). Overall, only 20 to 40% of sequences affiliated with well-characterized groups of bacteria can be assigned to known genera. Of the genera in the well-defined groups, the three most abundant at 100% assignment confidence are *Burkholderia* (class *Betaproteobacteria*), *Pseudomonas* (class *Gammaproteobacteria*), and *Chitinophaga* (class *Sphingobacteria*), which constitute 2.7, 1.6, and 1.0% of all the sequences, respectively.

Although recognized as the essential basis of bacterial systematics (115), the genus rank has not been well defined. In essence, though, genera tend to consist of species that share major phenotypic characteristics that differentiate them from species of related genera. The consequence of being able to assign only 10 to 21% of sequences to known genera is that the broad characteristics of most of the soil bacteria are not known.

ABUNDANCE OF DIFFERENT PHYLA

16S rRNA genes from soil bacteria are affiliated with at least 32 phylum-level groups. The contributions that members of different phyla make to the different soil bacterial communities vary (Fig. 1). The dominant phyla in the libraries are *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, *Planctomycetes*, *Gemmatimonadetes*, and *Firmicutes* (Table 4). Members of these nine phyla make up an average of 92% of soil libraries (normalized for the size of the individual libraries). Although there are at least 52 bacterial phyla (88), and 24 are recognized by *Bergey's*

TABLE 4. Contribution of 16S rRNA and 16S rRNA genes from members of different phyla and subphylum groups (class, subdivision, or subclass) to soil bacterial communities[a]

Phylum	Subphylum	Mean contribution (%)	Range (%)
Acidobacteria	Subdivision 1	3.3	0–14.4
	Subdivision 2	0.5	0–3.3
	Subdivision 3	1.8	0–4.9
	Subdivision 4	7.7	0–35.0
	Subdivision 5	0.4	0–2.2
	Subdivision 6	4.5	0–12.8
	Subdivision 7	1.5	0–7.4
Actinobacteria	*Acidimicrobidae*	2.4	0–8.9
	Actinobacteridae	4.7	0–18.3
	Rubrobacteridae	5.6	0–24.8
Proteobacteria	*Alphaproteobacteria*	18.8	1.8–43.1
	Betaproteobacteria	10.0	2.1–31.1
	Gammaproteobacteria	8.1	1.1–34.1
	Deltaproteobacteria	2.3	0–10.1
	Epsilonproteobacteria	0.04	0–0.8
Verrucomicrobia	*Verrucomicrobiae*	0.03	0–0.7
	Spartobacteria	6.3	0–21.1
	Subdivision 3	0.5	0–4.7
	Subdivision 4	0.2	0–1.1
Bacteroidetes	*Flavobacteria*	0.4	0–3.2
	Sphingobacteria	4.6	0–15.9
Firmicutes	*Bacilli*	1.6	0–7.0
	Clostridia	0.2	0–1.4
Chloroflexi		3.2	0–15.8
Planctomycetes		2.0	0–7.8
Gemmatimonadetes		2.0	0–3.7
Other groups		5.2	2.2–9.9
Unknown		2.4	0–12.6

[a] Only the 21 libraries with ≥90 clones were included in this survey (total, 2,920 clones).

TABLE 5. Estimates of abundance of the members of different bacterial groups made by cultivation-independent methods other than clone library analysis

Source (site designation)[a]	Method[b]	No. of members of indicated group[c]										Reference(s)
		ACI	ACT	ALF	BET	GAM	DEL	VER	BAC	FIR	PLA	
Cropland, Italy	FISH		4	20	15	5			8	6	5	18
Organic soil, Norway	FISH		<1	2–5	<1–5	<1	5–8		<1		3–7	20
Mineral soil, Germany	FISH		<1	7–10	<1–4	<1	4–7		<1		3–7	20
Tundra, Russia	FISH		4	1	12	3			8	6		64
Cropland, Germany	FISH			3	10	25			2			102
Forest, Germany	FISH		<1	7	<1	1	3		<1		7	118
Tilled cropland, United States (CT)	rRNA	<1–9	5–22	18–41	2–6			<1–3	<1		5–7	15, 16
Tilled cropland, United States (NI)	rRNA		7	26	4	3						15
No-till cropland, United States (AF)	rRNA		7	26	4	3						15
No-till cropland, United States (NT)	rRNA		11	31	5	3						15
Abandoned field, United States (HCS)	rRNA	1–3	9–27	7–33	1–5			1–3	<1		3–9	15, 16
Abandoned field, United States (LS)	rRNA	4	10	38	1			2	<1		13	16
Tilled grassland, United States (HCST)	rRNA	2	14	4	<1			<1	<1		2	16
Meadow, United States (NCS)	rRNA	<1–3	9–18	12–41	1–9	4		1–3	1–2		4–12	15, 16
Tree plantation, United States (PL)	rRNA	1	7	16	1			<1	<1		3	16
Meadow, The Netherlands	rRNA		19	22						48		39
Desert, United States	qPCR	19	5	7	4				1	4		40
Forest, United States	qPCR	14	5	14	5				1	3		40
Prairie, United States	qPCR	23	6	9	8				1	6		40

[a] The site designations are those used by authors to identify particular sources within studies with multiple soil samples.

[b] FISH, counting of cells in soil samples with group-specific oligonucleotide probes; rRNA, estimation of abundance of rRNA in total rRNA by hybridization with group-specific oligonucleotide probes; qPCR, quantitative PCR estimate of 16S rRNA genes using group-specific assays, relative to estimates of total bacteria using *Bacteria*-specific assays.

[c] ACI, phylum *Acidobacteria*; ACT, phylum *Actinobacteria*; ALF, class *Alphaproteobacteria*; BET, class *Betaproteobacteria*; GAM, class *Gammaproteobacteria*; DEL, class *Deltaproteobacteria*; VER, phylum *Verrucomicrobia*; BAC, phylum *Bacteroidetes*; FIR, phylum *Firmicutes*; PLA, phylum *Planctomycetes*.

Manual (48), soils seem to be dominated by only a small number of these.

Members of the phyla *Proteobacteria* and *Acidobacteria* are the most abundant soil bacteria, as judged by the occurrence of 16S rRNA and 16S rRNA genes that are assignable to these groups (Table 4). All of the libraries surveyed contained some sequences assignable to these two phyla. The other dominant phyla are not found in all libraries, but this is likely to be a consequence of library size. Given that there is variation in the contribution of different phyla and classes, abundances at the lower end of any range may mean that no sequences are detected if the library is too small. In addition to representatives of the nine major phyla, members of a number of other phylum-level lineages, such as *Chlamydiae*, *Chlorobi*, *Cyanobacteria*, *Deinococcus-Thermus*, *Fibrobacteres*, *Nitrospirae*, BRC1, NKB19, OP10, OP11, OS-K, SC3, SC4, termite group I, TM6, TM7, WS2, and WS3, are present in the global data set. Some members of a few of these phyla are quite well studied, but in general very little is known about the soil-inhabiting members of these groups. Most of these phyla are virtually unstudied and have few or no known pure culture representatives from soils (74).

A number of studies using methods other than analysis of libraries have estimated the contribution of members of different bacterial groups to the microbial population of soils (Table 5). These studies support the general trends observed in clone libraries (Fig. 1, Table 4) that *Alphaproteobacteria*, *Acidobacteria*, and *Actinobacteria* are often abundant in soils and that members of *Bacteroidetes*, *Firmicutes*, and *Planctomycetes* are generally less abundant (Table 5). They also support the observation that the estimated abundance of the major phyla varies between different soils (or samples). It is not possible to state to what degree the variations are method based. Fluorescence in situ hybridization (FISH) and other hybridization methods may detect bacteria other than the intended target group, or the phylogenetic coverage of oligonucleotide probes may not be comprehensive. The same applies to oligonucleotides designed for quantitative PCR approaches. Detection of extracted rRNA is affected by ribosome levels in bacteria, while clone library compositions are influenced by PCR steps and by *rrn* copy number. The results obtained with all the methods are affected by the physical nature of bacterial cells, which may vary between groups and under different conditions, affecting oligonucleotide probe permeability and successful nucleic acid extraction.

PHYLUM LEVEL DIVERSITY

Members of the phylum *Proteobacteria* make up an average of 39% (range, 10 to 77%) of libraries derived from soil bacterial communities (Fig. 1). Most soil-dwelling members of the phylum *Proteobacteria* can be classified within the classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria* (Table 4). The phylum *Proteobacteria* currently contains some 528 named and described genera (48), but the number of proteobacterial sequences that can be confidently assigned to known genera is relatively low. Depending on the level of confidence used, only 19 to 36% of the proteobacterial sequences can be assigned to a known genus (Table 3), indicating that many proteobacterial groups still remain to be described and named. Libraries from soils reveal the existence of lineages not affiliated with known isolates (e.g., see references 5, 37, and 77). Given the extent of physiological diversity within the phylum, no guesses as to their general metabolism can be made. At present, research interest seems to be directed toward the as-yet-uncultured groups within phyla such as *Acidobacteria* and *Verrucomi-*

crobia, which constitute smaller parts of the soil bacterial community; the uncultured proteobacteria have been largely ignored. Members of some cosmopolitan family or order level groups of the *Alphaproteobacteria* and *Gammaproteobacteria* without named and described genera have been isolated but are not yet formally named (29, 60, 94). 16S rRNA genes affiliated with these isolates have been found in most of the libraries listed in Table 1.

Members of the phylum *Acidobacteria* make up an average of 20% (range, 5 to 46%) of soil bacterial communities (Fig. 1). The phylum *Acidobacteria* is divided into at least eight subdivisions that may have class level rank (56). Three of these are particularly abundant in soils: subdivisions 1, 4, and 6 (Table 4). There are, however, only three formally described genera in the phylum (48). Members of subdivision 1 have been shown to be readily culturable. Kishimoto and Tano (62) originally cultured eight isolates, one of which was named *Acidobacterium capsulatum* (63). Since then, at least 99 further isolates that span the phylogenetic breadth of the subdivision have been reported (29, 52, 58, 60, 93, 94, 103). A few isolates of members of subdivisions 2, 3, and 4 have been obtained in pure culture (29, 60, 78, 92, 94). All isolates appear to be aerobic heterotrophs, but this may be a result of the cultivation strategies employed to date. The phylogenetic breadth of subdivisions 3 and 4 in particular is much greater than that covered by the few isolates (e.g., see references 5, 7, 50, 56, 71, 87, and 121). To date, no isolates of subdivisions 6 and 7 have been reported, even though members of these subdivisions are common in soils (Table 4). Members of subdivision 6 may be aerobes, since they were not detected in a permanently anoxic soil system (116) and colonies have recently been found to form on plates incubated under air (K. E. R. Davis and P. H. Janssen, unpublished data). The phylogenetic depth of the phylum *Acidobacteria* is nearly as great as in the phylum *Proteobacteria*, and the different class rank subdivisions may contain bacteria with very different physiologies (31, 56, 75). For example, the only known members of subdivision 8 are obligate anaerobes, with two different basic physiologies, which contrast with the known aerobes of subdivisions 1 to 4 (23, 63, 70, 93).

Members of the phylum *Actinobacteria* make up an average of 13% (range, 0 to 34%) of soil bacterial communities (Fig. 1). The phylum *Actinobacteria* contains three subclasses that are common in soil: *Actinobacteridae*, *Acidimicrobidae*, and *Rubrobacteridae* (Table 4). For the purposes of this synthesis, the subclasses are used as subphylum groupings. The majority of described genera of the phylum *Actinobacteria* are within the subclass *Actinobacteridae*. This group consists of some 158 genera, many of which are well known and well studied (48). Although members of the subclass *Actinobacteridae* have been extensively investigated, only 26 to 47% of sequences could be assigned to described genera and there remains considerable scope for the isolation of novel members of this group, especially new rare genera that may yield novel bioactive compounds (67).

In addition to members of the subclass *Actinobacteridae*, soils contain many members of the less-studied subclasses *Rubrobacteridae* and *Acidimicrobidae* (Table 4). To date, there are no validly named and described members of the subclass *Acidimicrobidae* from soil, and only five isolates from soil been reported (29, 60). The only named and characterized members of this subclass are *Acidimicrobium ferrooxidans* and *Ferromicrobium acidophilus*, which are ferrous-iron-oxidizing acidophiles, and *Microthrix parvicella*, an as-yet-uncultured filamentous bacterium found in activated sewage sludge (6, 11, 22). Only *Acidimicrobium ferrooxidans* is currently recognized by *Bergey's Manual* (48).

Two genera of aerobic heterotrophs from soil in the subclass *Rubrobacteridae* have been described. These are *Solirubrobacter* and *Conexibacter*, each represented by one species with one strain each (80, 100). Twenty further isolates, some phylogenetically distant from these two genera, have been obtained from soil (29, 58, 60, 94, 96). These are all aerobic heterotrophs. The few other members of this subclass are aquatic thermophiles of the genera *Rubrobacter* and *Thermoleophilum* (19, 21, 106, 117). Overall, there are many lineages without cultured representatives in all three subclasses of soil-inhabiting actinobacteria, especially in the subclasses *Rubrobacteridae* and *Acidimicrobidae*, but also some in the subclass *Actinobacteridae* (e.g., see references 5, 50, 54, 73, 79, 89, and 90). Recently, members of some of the previously uncultured lineages of the *Actinobacteridae* have been shown to be culturable (60). The phylogenetic depth of the phylum *Actinobacteria* appears to be lower than that of other major phyla, but the degree of phenotypic diversity in this phylum is high (31, 47). The as-yet-uncultured actinobacterids can be expected to be aerobic heterotrophs, but the subclasses *Rubrobacteridae* and *Acidimicrobidae* may yet contain other metabolic types.

Members of the phylum *Verrucomicrobia* make up an average of 7% (range, 0 to 21%) of soil bacterial communities (Fig. 1). The phylum has been divided into five major class level subdivisions (56). The major group of *Verrucomicrobia* found in soil is the class *Spartobacteria* (Table 4), which is the name proposed for subdivision 2 of *Verrucomicrobia* (97). *Chthoniobacter flavus* is the first named cultured isolate of the class *Spartobacteria* (58, 97). *C. flavus* and a further nine isolates belong to at least two genera within the family *Chthoniobacteraceae* in the class *Spartobacteria* (96). These are all aerobic heterotrophs. The class *Spartobacteria* also contains bacterial symbionts of nematodes in the family *Xiphinematobacteraceae* (112), and the cloned sequences detected in soils may therefore have come from free-living or symbiotic bacteria. The 10 isolates of the class *Spartobacteria* do not cover the phylogenetic breadth of the class (96), and many unrepresented lineages (e.g., see references 5 and 56), indicative of novel genera and families, remain to be cultured. There is no indication of what the physiologies of members of those families could be. Only six isolates of subdivision 3 of the phylum *Verrucomicrobia* have been obtained (60, 96). These are aerobic heterotrophs, but they also do not cover the full phylogenetic breadth of the subdivision and so do not yet give a complete picture of the phenotypes of members of this group (96).

Members of the phylum *Bacteroidetes* make up an average of 5% (range, 0 to 18%) of soil bacterial communities (Fig. 1). There is some evidence suggesting that members of this phylum may be underrepresented in libraries of PCR-amplified 16S rRNA genes (27, 34). Even so, members of the class *Sphingobacteria* of the phylum *Bacteroidetes* are common in soils (Table 4). Some members of this group are aerobes, while others are anaerobes or facultative anaerobes, and so the species composition of members of this class within a soil may depend on oxygen levels or the amount of variation in oxygen

availability. Members of the class *Flavobacteria* are less common (Table 4), and members of the third class, *Bacteroidetes*, seem to be absent from soils. Of all the sequences affiliated with the phylum *Bacteroidetes*, 34 to 62% could be assigned to known genera, including *Chitinophaga*, *Flavobacterium*, *Hymenobacter*, and *Pedobacter*, suggesting that this is one of the few groups of dominant soil bacteria that is readily culturable. However, given the high diversity of soil bacteria, we should not be surprised if many novel genera, albeit in low abundance, exist in soil. Lineages without cultured representatives have been detected (e.g., see references 72 and 77).

Members of the phylum *Chloroflexi* make up an average of 3% (range, 0 to 16%) of soil bacterial communities (Fig. 1). The phylum *Chloroflexi* consists of perhaps eight candidate classes, and the phylogenetic depth is comparable with the phylum *Proteobacteria* (31, 88). Only eight described genera are known, and these are not evenly distributed within the classes (48). Rappé and Giovannoni (88) and Hugenholtz et al. (56) recognize the *Thermomicrobia* as a class of the phylum *Chloroflexi*, with one described genus, but Garrity et al. (48) accord the *Thermomicrobia* separate phylum status. The diversity of phenotypes in this phylum is high, even among the relatively few isolates that have been cultured to date (88). 16S rRNA genes from uncultured soil bacteria belonging to the phylum *Chloroflexi* are affiliated with a number of the candidate classes and so may display quite different physiologies (88). Only one isolate from soil has been reported (29). It is a filamentous aerobic heterotroph, but no conclusions can be drawn yet about the general properties of soil chloroflexi. There is evidence that other members of this group are culturable (Davis and Janssen, unpublished data).

Members of the phylum *Planctomycetes* make up an average of 2% (range, 0 to 8%) of soil bacterial communities (Fig. 1). The planctomycetes are a group of budding bacteria that lack peptidoglycan and possess membrane-bound intracellular compartments (44). The phylogenetic depth within the group is sufficient to suggest that the phylum could be composed of at least three classes (31, 88). One of these could consist of bacteria such as those of the candidates *Brocadia* and *Kuenenia*, which are involved in anaerobic nitrification (88). The other two major groups are defined by the relatively well studied genera classified in the class *Planctomycetacia* (48) and by sequences affiliated with the WPS-1 lineage (82). Soil bacteria are affiliated with all three major groups, and there are many lineages without any cultured representatives (e.g., see references 50, 68, and 88). Most isolates of this phylum are from aquatic sources, and it is not clear whether these are physiologically and genetically good models for soil planctomycetes. Isolates from soil, including a few members of the WPS-1 lineage, have been reported (29, 60, 114), but these do not represent the full phylogenetic breadth suggested by the sequences detected in soils.

Members of the phylum *Gemmatimonadetes* make up an average of 2% (range, 0 to 4%) of soil bacterial communities (Fig. 1). The phylum *Gemmatimonadetes* contains only one named and described species, *Gemmatimonas aurantiacus* (120). This bacterium is a gram-negative aerobic heterotroph isolated from an anaerobic-aerobic sequential batch reactor and belongs to subdivision 1, also known as the class *Gemmatimonadetes* (48, 120). Four isolates from soil have been obtained (29, 60). They too belong to subdivision 1 and display an aerobic, heterotrophic phenotype. Soil-inhabiting representatives of the phylum are found through most of the phylogenetic breadth of the group, which may contain a number of discrete class rank taxa (81, 88, 120). The diversity of general physiologies of this group remains to be ascertained.

Members of the genera *Bacillus* and *Clostridium* have long been considered to be common members of the soil bacterial community, but the classes *Bacilli* and *Clostridia* of the phylum *Firmicutes*, together comprising of some 214 genera, including *Bacillus* and *Clostridium* (48), contribute only a mean of 2% (range, 0 to 8%) to the libraries (Fig. 1). It is possible that members of this group are underrepresented in libraries because the cells or spores may be difficult to lyse and so are not detected in PCR-based analyses that rely on DNA extraction from soil. Until evidence for such a bias is available, members of this group must be considered to be relatively minor components of soil bacterial communities. They may, however, be locally abundant, such as in a grassland soil in The Netherlands (38, 39). Of the sequences affiliated with the phylum *Firmicutes* in the 32 libraries analyzed, 17 to 52% could be assigned to known genera, suggesting that a number of new genera remain to be isolated, named, and described.

This review deals with members of the domain *Bacteria*, but members of the domain *Archaea* have also been detected in soils (10, 12, 17, 61, 111), although their abundance is generally low (17, 84, 99). These studies also reveal the presence of high-rank taxa of the domain *Archaea* with no cultured representatives. Some of these soil archaea may prove to have unexpected physiologies (42), and they appear to be culturable (99).

CHALLENGES AND GOALS

There is considerable variability in the abundance of members of different phyla and classes in different soils, judged by the abundance of 16S rRNA or 16S rRNA genes in libraries. It is not yet clear to what extent the variations are systematic, in response to conditions in the soil environment, and to what degree method-induced artifacts impact the data. The number of different biological, chemical, and physical factors that may influence the abundance of different bacterial groups could be very large. It has been suggested that the abundance of verrucomicrobia is influenced by soil moisture (14), and the abundance of members of subdivision 1 of the acidobacteria appears to be controlled by soil pH (93). It is not known whether the abundance of members of other high-rank taxa is controlled by single, readily identifiable factors. The degree of phenotypic variation within some of the groups must mean that the total abundance of a particular group may not change as much as the representation of species within that group, and so the abundance of such phenotypically diverse groups cannot be expected to be controlled by single variables.

Regardless of whether one is interested in functional or phylogenetic groupings, it is clear that the physiologies and characteristics of the poorly studied groups of soil bacteria must be of interest to soil microbiologists (49, 55, 85, 88, 122). Those interested in functions will want to identify all the major contributors to that function and will not want to disregard the possibility that bacteria among the poorly characterized part of the community are involved. The complexity of soil microbial

communities means that metagenomic approaches to studying soil bacteria and assembling genomes of uncultured bacteria to understand their physiologies seem impractical at present (110). Although the assignment of functions and associated genes to phylogenetic markers is possible in the absence of cultures (49, 69), it can be achieved more easily with cultured bacteria.

Culturing of the total diversity of species, estimated at 10^4 to 10^6 per 10-g soil sample (28, 46, 109), currently seems an unreachable goal, given that such large numbers of isolates are not routinely cultured and identified and that the rarer species can be expected to be difficult to find among the colonies that do form. An initial objective should be to obtain a range of isolates from representatives of members of the different phyla and classes and to determine to what extent there is functional and genetic diversity within these groups. This will give us an initial overview of the potential roles of different soil bacterial groups and will also enable us to learn the tricks required to culture their more recalcitrant relatives. This strategy has been successfully applied to verrucomicrobia and acidobacteria (93, 96). At the same time, genome sequences of selected isolates will help fill in the bacterial genome tree (55).

Some advances in culturing soil bacteria have been made in the last few years (29, 58, 60, 93, 94, 96, 103, 107, 119). These recent advances mean that it is probably incorrect to speak of the majority of bacterial species in soil as being unculturable. Instead, we should be aware that isolating them will take patience and careful selection of appropriate strategies. Many of the isolates of rarely isolated groups are very slow growing and are difficult to maintain in the laboratory (29). The formation of visible colonies requires weeks or months rather than hours or days. Although the growth rates are low, they are still much higher than their likely growth rates in soil, where cells may divide only a few times per year (53). It is likely that the few isolates of *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, *Gemmatimonadetes*, *Chloroflexi*, *Acidimicrobidae*, and *Rubrobacteridae*, as well as many of the isolates of the better-studied *Proteobacteria*, *Bacteroidetes*, and *Actinobacteridae*, are actually the more readily cultured representatives of these groups. To isolate type strains and a representative collection of related strains, and to deposit them in culture collections as required for the valid description of new species (101), will require great dedication and a high level of commitment and expertise from soil microbiologists and from culture collections. Successful approaches to culturing these organisms require patience, but the outcomes are immensely satisfying to microbiologists who enjoy the challenge and savor the reward of observing the colony on the plate, seeing the cells of the pure culture under the microscope, elucidating the bacterium's physiology, or releasing its genome sequence into public databases.

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