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# Bacterial Diversity in Adirondack Mountain Lakes as Revealed by 16S rRNA Gene Sequences

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**Bacterial communities of seven lakes in the Adirondack Mountains of New York State were characterized by amplification and sequencing of 16S ribosomal DNA. Analysis of over 100 partial sequences revealed a diverse collection of lineages, largely of the class *Proteobacteria* (19% alpha subdivision, 31% beta subdivision, and 9% gamma subdivision), the phylum *Cytophaga-Flavobacteria-Bacteroides* (15%), and the order *Actinomycetales* (18%). Additionally, a number of the sequences were similar to those of the order *Verrucomicrobiales*. However, few of the sequence types are closely related to those of characterized species. The relative contributions of the groups of sequences differed among the lakes, suggesting that bacterial population structure varies and that it may be possible to relate aquatic bacterial community structure to water chemistry.**

Aquatic bacteria play crucial roles in decomposition, food chains, and biogeochemical cycling, yet they are largely uncharacterized due to their small size, the limited range of morphologies, and the difficulties in obtaining pure cultures that are representative of natural populations. The promise that was seen for rRNA studies of natural microbial communities (15) has been borne out in a rapidly increasing literature, and apparently dominant marine organisms have been identified phylogenetically (3, 9). Unfortunately, investigations of freshwater lake ecosystems have been far less numerous than those of marine environments, and equivalent insights have not been made.

The Adirondack Mountain region has been affected by deposition of acids and metals from fossil fuel combustion in the Ohio Valley. Biological effects of acidification ranging from changes in phytoplankton communities to complete loss of fish have been documented, but effects of acidification on bacterial communities are unknown (1). This study ultimately will assess the impacts of anthropogenic acidification on aquatic bacterial communities and may also identify indicator species useful for the assessment of acidification and recovery of lakes.

The bacterial communities of seven lakes in the southwest region of the Adirondacks were sampled for rRNA gene sequence analysis as a preliminary to applying in situ hybridization for detailed analyses of community structure. Depth-integrated epilimnetic water samples were collected above the maximum lake depth during summer stratification. Samples were maintained on ice and concentrated in aliquots of 250 to 750 ml on sterile 0.2-μm-pore-size filters (Gelman Supor-200 or Millipore Durapore GV) by using an autoclaved apparatus rinsed with sample water immediately before use. Filters were stored at −80°C, each in 1 ml of preservation buffer (22).

Genomic DNA was extracted by using a lysozyme-sodium dodecyl sulfate-proteinase K protocol based on that of Giovannoni et al. (8). Bacterium-specific primers fD1 and rP2 (21) (*Escherichia coli* positions 8 to 23 and 1492 to 1393; reference 4) were used for PCR (95°C for 1 min, 54°C for 1 min, and 72°C for 2 min for 35 cycles and a final extension at 72°C for 7 min). Negative (deionized water for the template) and positive (plasmid containing a 16S rRNA insert) control reactions were run with each set of amplifications.

Amplification products were size fractionated and cloned (TA cloning kit; Invitrogen). Partial insert sequences (approximately 350 nucleotides) were obtained (Sequenase 2.0 [USB] or AmpliCycle [Perkin-Elmer]) from a single primer (*E. coli* positions 522 to 536). When entire insert sequences were obtained, two vector sequence primers and five internal rRNA primers were used (21). Representative sequences have been assigned GenBank accession numbers U85098 to U85191.

rRNA gene sequences (designated ACK) were compared to the Ribosomal Database Project (RDP) (13) SSU_Prok data set (release 5.0) and secondary structures and manually aligned by using Genetic Data Environment version 2.2 (17). Bootstrap-tested phylogenies were constructed for 228 positions (spanning *E. coli* positions 658 to 907) by neighbor joining of Kimura distances and by parsimony (PHYLIP 3.5; reference 6). Three apparently chimeric sequences were detected by using the RDP CHECK_CHIMERA facility and by comparing phylogenetic analyses of the 5′ and 3′ halves of the data set.

Nearest-neighbor sequences were identified from the RDP SSU_Prok data set (13) (Table 1), and Adirondack sequences were compared phylogenetically to RDP data that are representative of the domain *Bacteria* (Fig. 1). These comparisons do not give an exhaustive detailing of the nearest relatives of ACK sequences. Rather, they are an overview of the range of phylogenetic types and diversity found in the ACK data set.

This is the first report of an extensive ribosomal DNA sequence data set from freshwater lakes. A recurrent theme in environmental studies of rRNA phylogeny is the discovery of lineages that are well separated from those of previously described microorganisms. This study continues that trend (for example, the ACK-3 cluster, related most closely to the SAR11 group of marine sequences). Nevertheless, although there is great diversity among the ACK sequences recovered, they are mostly ascribable to members of the α, β, and γ subdivisions of the class *Proteobacteria* (19, 31, and 9%, respectively), the phylum *Cytophaga-Flavobacteria-Bacteroides* (15%), and the order *Actinomycetales* (18%). Even at this superficial level, the distribution of sequences seems different from those found in

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TABLE 1. Nearest RDP neighbors of ACK sequences[a]

| Major affiliation | Designation in Fig. 1 | ACK clone designation(s) | SIMILARITY_RANK nearest neighbor (reference) | Score(s) |
|---|---|---|---|---|
| *Cytophaga-Flavobacteria-Bacteroides* | ACK-M21 group | M21, M36, W12 | *Flectobacillus major* | 0.559–0.522 |
| | None | L4 | *Flectobacillus major* | 0.727 |
| | Subdivision II ACK group | C25, C70, M42 | *Cytophaga arvensicola* | 0.653–0.618 |
| | | C2, C99, DE12, M32, M44, M61 | *Flavobacterium ferrugineum* | 0.759–0.539 |
| | | DE64 | *Flexibacter sancti* | 0.671 |
| | None | DH12 | agg32 (5) | 0.407 |
| | | W3 | *Prevotella buccalis* | 0.405 |
| Relatives of *Verrucomicrobium* sp. | MC18 group | M6 | MC 17 (12) | 0.585 |
| | | DE41, DE36 | MC 31 (12) | 0.598, 0.553 |
| | None | DH1 | *Legionella jordansis* | 0.415 |
| | | DH7 | *Legionella* sp. strain LLAP-3 | 0.364 |
| | | M10 | Symbiont of *Solemya velum* | 0.386 |
| Relative of *Acidobacterium* sp. | None | C84 | *Acidobacterium capsulatum* | 0.649 |
| Alpha *Proteobacteria* | ACK-3 cluster | DE28, M13, M20, W4, W7 | SAR11 (9) | 0.452–0.391 |
| | | DE33, DH2, M22 | SAR95 (3) | 0.455–0.431 |
| | Alpha-2 ACK group | DE24, DE48, DE55, M37, SA8, W10 | *Beijerinckia indica* subsp. *indica* | 0.914–0.642 |
| | | DE11 | *Rhodopseudomonas viridis* | 0.464 |
| | | DE46 | *Afipia clevelandensis* | 0.753 |
| | None | DE34 | *Azospirillum* sp. | 0.568 |
| | | DE40 | *Azospirillum amazonense* | 0.619 |
| | | DH13 | *Caulobacter bacteroides* CB7 | 0.695 |
| Beta *Proteobacteria* | ACK-1 cluster | M46, L5[b] | *Burkholderia pickettii* K 550 | 0.741, 0.697 |
| | | C11, C21, C27, C28, C31, C4, C41[b], C45, C47, C85, GR2, GR6, L6[b], L7, M24, M41, M47, M51, M55, M7, SA6, SA9 | *Burkholderia solanacearum* | 0.713–0.613 |
| | ACK-2 group | C66, C78 | *Brachymonas denitrificans* AS-P1 | 0.876, 0.809 |
| | | SA4 | *Rhodoferax fermentans* FR2 | 0.843 |
| | | C7, C87 | *Variovorax paradoxus* IAM 12373 | 0.868, 0.809 |
| | None | C86 | *Burkholderia pickettii* K 550 | 0.605 |
| | | C30[b] | *Burkholderia pseudomallei* | 0.738 |
| | | M50 | *Methylophilus methylotrophus* AS1 | 0.708 |
| Gamma *Proteobacteria* | Enteric cluster | SA1, SA7 | *Escherichia coli* K-12 strain MG1655 | 0.937, 0.675 |
| | *Xanthomonas* cluster | DE58, DE52 | *Xanthomonas maltophilia* | 0.917, 0.903 |
| | None | M9 | *Ectothiorhodospira shaposhnikovii* | 0.621 |
| | | DE72 | *Legionella pneumophila* ATCC 33152 | 0.725 |
| | | DH11 | Symbiont of *Anodontia phillipiana* gill | 0.603 |
| | | DE70 | Symbiont of *Codakia costata* gill | 0.545 |
| *Actinomycetales* | ACK-4 group | C58, DE26, M2, M3 | *Arthrobacter* sp. strain H1 | 0.556–0.465 |
| | | C65, C67, C68, M1[b], M15, M19, W8 | *Corynebacterium mediolanum* | 0.589–0.544 |
| | | C53, M17 | *Corynebacterium renale* | 0.610, 0.588 |
| | | SA3 | *Frankia* sp. strain AcN14a | 0.544 |
| | | DH8 | *Frankia* sp. strain ArI4 | 0.506 |
| | | C3 | *Micrococcus luteus* Hucker S66 | 0.587 |
| | None | C101 | *Streptomyces ambofaciens* | 0.541 |
| Chloroplasts and cyanelles | Chloroplasts and cyanelles | M44 | *Astasia longa* chloroplast strain CCAP 1204-17a | 0.433 |
| | | DE32 | *Chlamydomonas moewusii* chloroplast strain UTEX 97 | 0.735 |
| | | M11 | *Ochromonas danica* chloroplast strain SAG 933-7 | 0.51 |
| | | C60, M8 | *Olisthodiscus luteus* chloroplast | 0.595, 0.551 |
| | | C81 | *Palmaria palmata* chloroplast | 0.356 |
| | | C39, W11 | *Pyrenomonas salina* chloroplast | 0.739, 0.703 |
| | | M58 | Symbiont of *Photoblepharon steinetzi* | 0.392 |

[a] Sequences were sorted by phylogenetic, not nearest-neighbor, analysis. These two features are generally compatible, but note that nearest neighbors of DH1, DH7, and M10 are in the γ subdivision of the class *Proteobacteria*. However, these are not close neighbors. Clusters are as shown in Fig. 1 and do not necessarily indicate a high percentage of identity or exclusion of unlisted reference sequences. Sample sites (ACK clone designations): Carry Pond (C), Dart's Lake (DE and DH), Grass Pond (GR), Limekiln Lake (L), Moss Lake (M), Sagamore Lake (SA), and Windfall Pond (W).

[b] Long sequence.

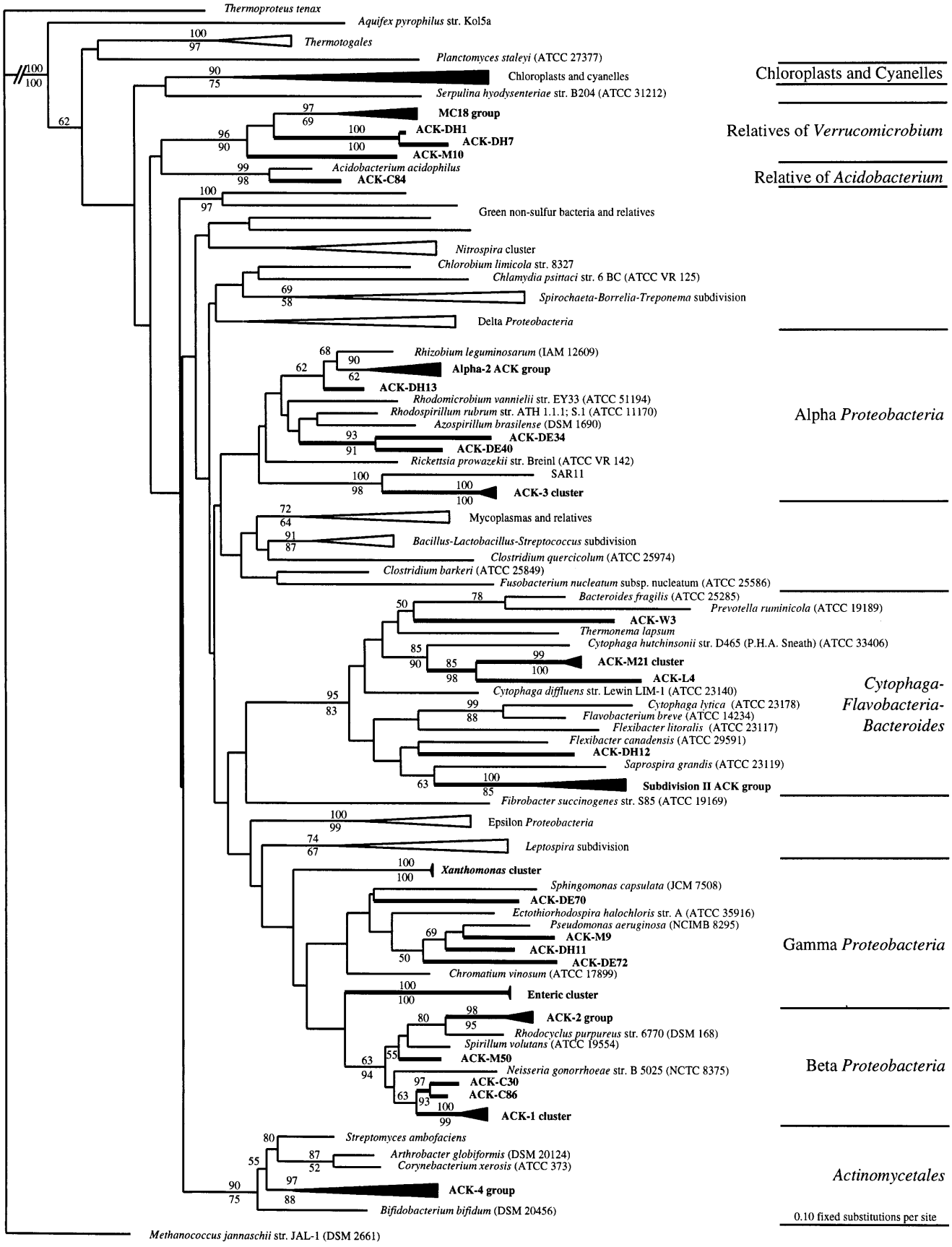


FIG. 1. Overview of phylogenetic diversity of 109 ACK clones. Triangles represent multiple sequences. Filled triangles indicate clades containing ACK sequences and not necessarily excluding RDP sequences. Bootstrap values above 50% are shown above branches for distance trees and below branches for maximum-parsimony trees.

oceanic (14) and subterranean waters (16) and soils (2, 18); detailed comparisons are beyond the scope of this report. No representation of the order *Planctomycetales* was seen, which is perhaps surprising given the widespread occurrence of these organisms in freshwater (19). Relatives of the prosthecate freshwater bacteria *Verrucomicrobium spinosum* and *Prosthecobacter* sp. (10, 20) have been found. Interestingly, members of this recently defined monophyletic group have also been detected in ribosomal DNA studies of geographically widespread forest and pasture soils (11, 12) and the Pacific Ocean (7). This clade seems worthy of consideration in future hybridization probe studies of bacterial communities.

This program is aimed at characterization of the biological components of a range of freshwater Adirondack lake ecosystems, with the goal of relating environmental parameters to community composition. Although the sampled lakes are geographically close to each other, their chemical characteristics and biological responses to acidic deposition differ markedly because of complex biological, chemical, and physical processes in the individual watersheds and lakes. Acidification can affect most trophic levels in Adirondack lakes with various degrees of severity, but it is not known whether bacterial communities are responsive to this stress or are tolerant and uniform between sites. Uniformity might reflect the physical proximity of the study sites or the existence of a generalist lake community. The extents of variation between microbial communities due to habitat or geography are not known. The data obtained in this study indicate that there are commonalities between these Adirondack lakes but appear also to demonstrate lake-specific differences. As an example, the 19 α-proteobacterial sequences include 11 of the 26 Dart Lake sequences but none of the 31 from Carry Pond. This suggests that the bacterial community compositions of these lakes are not homogeneous and potentially are influenced by environmental parameters. However, the Adirondack α-proteobacterial sequences are diverse and polyphyletic (Fig. 1 and Table 1), a feature that would not have been detected by using oligonucleotide probes that encompass such broad phylogenetic groups. Patterns of community structure are also likely to exist at more refined levels, and while detailed comparisons between lakes cannot be made directly from this sequence data set (due to low numerical representation of the subclusters), tailored oligonucleotide hybridization probes will be used to assess this. Of course, design of probes specific for these groups was not possible without prior sequence description of the unknown bacterial community members. Thus, this data set establishes a foundation for that work and for the study of freshwater bacterial communities in general.

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## ADDENDUM IN PROOF

Several representative sequences derived from an arctic freshwater lake have been published recently (Bahr et al., Aquat. Microb. Ecol. **11:**271–277, 1996) under GenBank accession numbers U76088 to U76105.

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