Methanotrophic bacteria play a major role in the global carbon cycle, degrade xenobiotic pollutants, and have the potential for a variety of biotechnological applications. To facilitate ecological studies of these important organisms, we developed a suite of oligonucleotide probes for quantitative analysis of methanotroph-specific 16S rRNA from environmental samples. Two probes target methanotrophs in the family *Methylcoccaecae* (type II methanotrophs) as a group. No oligonucleotide signatures that distinguish between the two genera in this family, *Methylocystis* and *Methylosinus*, were identified. Two other probes target, as a single group, a majority of the known methanotrophs belonging to the family *Methylcoccaceae* (type I/X methanotrophs). The remaining probes target members of individual genera of the *Methylcoccaceae*, including *Methylbacter*, *Methylobo-lomonas*, *Methylomicrobium*, *Methyllococcus*, and *Methylocaldum*. One of the family-level probes also covers all methanotrophic endosymbionts of marine mollusks for which 16S rRNA sequences have been published. The two known species of the newly described genus *Methylosarcina* gen. nov. are covered by a probe that otherwise targets only members of the closely related genus *Methylomicrobium*. None of the probes covers strains of the newly proposed genera *Methylocella* and “*Methylothermus*,” which are polyphyletic with respect to the recognized methanotrophic families. Empirically determined midpoint dissociation temperatures were 49 to 57°C for all probes. In dot blot screening against RNA from positive- and negative-control strains, the probes were specific to their intended targets. The broad coverage and high degree of specificity of this new suite of probes will provide more detailed, quantitative information about the community structure of methanotrophs in environmental samples than was previously available.

Methanotropic bacteria are ecologically and technologically important because they comprise a critical link in the global carbon cycle, act as N₂ fixers and ammonia oxidizers, degrade a wide array of organic contaminants, and have biotechnological potential for single-cell protein production and novel enzyme functions (34, 43). Methanotrophs are interesting biologically because they are physiologically and phylogenetically unique. With the exception of two recent isolates (8, 24), all known methanotrophs belong to two monophyletic families: type I/X methanotrophs belong to the family *Methylcoccaecae* within the γ-Proteobacteria, and type II methanotrophs belong to the family *Methylocystaceae* within the α-Proteobacteria (10, 11, 14). For convenience and clarity, we will refer to the former as γ-methanotrophs and to the latter as α-methanotrophs when identifying them phylogenetically. No other phylogenetic clade is known to use CH₄ as a sole C and energy source (34). Hence, methanotrophs provide a striking example of a direct correspondence between physiology and phylogeny, making it possible to link process measurements with molecular phylogenetic approaches in situ (15, 17).

Although 16S rRNA-based phylogenies have been used effectively to resolve long-standing confusion over methano-
troph taxonomy (13, 14), a comprehensive suite of 16S rRNA-targeted oligonucleotide probes for the methanotrophs has proven difficult to design (9, 34). Some probes have been useful in monitoring CH₄ enrichment cultures (9, 37) or quantifying undifferentiated groups of diverse methylotrophs, including nonmethanotrophs, in environmental samples (52). However, the probes developed to date either are not specific to methanotrophs (36, 56) or fail to cover a large proportion of known methanotrophs (9, 34). Moreover, due to substantial diversity among the γ-methanotrophs that has been discovered in the past 4 years, such as the genera *Methylosphaera* (12), *Methylocaldum* (7), and *Methylosarcina* (58), many of these organisms have escaped detection by earlier probes.

To facilitate ecological studies of methanotroph communities, we designed a new suite of oligonucleotide probes and optimized them for quantitative hybridization analysis of 16S rRNA from specific groups of methanotrophic bacteria. Our aim was to design a complementary suite of probes that would (i) target methanotrophs to the exclusion of closely related nonmethanotrophic bacteria, (ii) encompass a greater number and wider diversity of known methanotrophic bacteria than achieved previously, and (iii) allow specific detection of methanotrophs at both the family and genus levels.

**MATERIALS AND METHODS**

**Bacterial cultures.** The reference cultures used in this study were obtained from various sources, as indicated, and are available from either the National Collection of Industrial and Marine Bacteria (NCIMB, Aberdeen, United Kingdom) or the American Type Culture Collection (ATCC, Manassas, Va.). Ref-
ference strains include Methylosinus trichosporum OB3b (NCIMB 11131) and Methylobacterium Bath (NCIMB 11132) (both provided by J. C. Marr) and Methylibacterium luteus (NCIMB 11914; provided by R. Knowles). Methylobacterium marinus A45 (nonex tant culture; genomic DNA provided by A. A. DiSpirito), Methylophilacinum album BGS (NCIMB 11123; provided by G. M. King), Methylophilum rubra (NCIMB 11913) and Methylosphaerina S1 (NCIMB 11130) (both provided by J. D. Semrau), Methylocollodium gracile (NCIMB 11112; purchased from NCIMB), Cialobacter crescentus CB15A (ATCC 19089; provided by J. S. Pointdexter), and E. coli (K18) (ATCC 11775; from laboratory stock culture).

All methanotrophs were grown at 30°C, except Methylococcus capsulatus Bath and Methylocallodium gracile, which were grown at 45°C, in nitrate mineral salts medium with CH4 and CO2 at an initial headspace mixing ratio of 45:5:50 (CH4 to CO2 to air) (35). E. coli was grown in Luria-Bertani broth under standard conditions (53), and C. crescentus CB15A was grown in PYCM medium (27) at room temperature.

Sequencing of 16S rRNA genes. Because ambiguous and missing bases in several of the sequences available from GenBank hindered sequence comparison, we resequenced the 16S rRNA genes of Methylobacterium rubra NCIMB 11913, Methylibacterium luteus NCIMB 11914, Methylosphaerina S1 NCIMB 11130, and Methylibacterium marinus strain A45. Nearly complete (1,450-bp) sequences were obtained for both the sense and antisense strands of the 16S rRNA gene using 5′ Long Ranger gel and an ABI PRISM DNA sequencer (41).

Selection of reference sequences. Probes were designed based on reference 16S rRNA sequences available from GenBank (6) and the Ribosomal Database Project (RDP-II) (42), as well as resequencing of key laboratory strains (see Table 1 and Fig. 3). BLAST (GenBank) and Probe Match (RDP-II) database searches were used to assess the potential breadth and specificity of the probe sequences. The reference sequences were aligned with the probe sequences to determine the apparent range of coverage of the candidate probes relative to the abundance and diversity of known methanotrophs. The 16S rRNA sequences specified by accession numbers in Fig. 2 and 3 represent all those available in the databases for confirmed methanotrophic isolates at the time of analysis. With the exception of the methanotrophic endosymbionts of marine mollusks (see below), we did not include sequences obtained from cultures that had not been characterized phenotypically or that were obtained by PCR amplification of environmental DNA.

Only cultured isolates with published, genus-level phylogenetic data were assigned genus designations in Fig. 2 and 3. Three general groups of confirmed methanotrophs were placed under “other α-methanotrophs” (Fig. 2) or “other γ-methanotrophs” (Fig. 3): (i) strains clearly belonging to the α- or γ-methanotrophs, but lacking or having dubious generic affiliations because of insufficient phylogenetic and taxonomic information (for example, “Methylosphaerina” strain S1Z is clearly a γ-methanotroph [57] but has not been characterized at the genus level); (ii) isolates validly assigned to the genera Methylocella, Methylophaga, and Methyloarcula, for which we did not design genus-level probes because there were only one or two known representatives of each genus; and (iii) the methanotrophic endosymbionts of marine mollusks, which lack generic descriptions. Although the RDP-II database includes many of these endosymbionts, it is not clear whether they are of active interest to microbial ecologists and evolutionary biologists because there is strong phenotypic and phylogenetic evidence that they are γ-methanotrophs (19, 20, 25, 28).

All available methanotroph 16S rRNA sequences that met the criteria given above were included in our analysis, regardless of sequence quality. However, a number of sequences appeared to be affected by common sequencing errors, including transposition of bases and duplicated or omitted bases. Some errors could be confirmed because they violated the integrity of the secondary structure of the 16S rRNA molecule, but others could not because they occurred in unpaired loop positions. Because sequence errors make designing group-level probes very difficult, we developed specific criteria for disregarding unexpected mismatches between a probe and a target sequence. We deemed destabilization of secondary structure sufficient grounds for disregarding mismatches. Additionally, we considered any two of the following criteria sufficient: (i) the mismatch occurs in a low-quality sequence as indicated by ambiguous bases in >0.5% of the positions in the entire sequence; (ii) the mismatch results from an ambiguous or missing base in the probe target region; (iii) multiple sequences for the same strain disagree in the mismatch position, and the higher-quality sequence, as indicated by percent ambiguity, matches the probe; (iv) a multiple alignment of all the sequences representing the target group shows that the mismatch is not representative of the target group; (v) the mismatch occurs in a highly conserved position of the 16S rRNA molecule; (vi) the mismatch is consistent with a common sequencing error, such as the transposition of two bases or the repetition of the same base, that disagrees with several other related sequences.

Oligonucleotide probe design. The oligonucleotide probes developed and/or optimized in this study are depicted in Table 1. The mismatch in probe designation represents the forward position of the homologous base in the E. coli 16S rRNA gene. By use of the SEQUAL sequence editor in the Wisconsin Package (Genetics Computer Group, Madison, Wis.), 16S rRNA sequences (>1,300 bp) were aligned initially using the PILEUP function within the editor and then adjusted manually with secondary-structure considerations as described previously (2). With the help of computer-generated consensus sequences, the alignments were scanned visually for signature sequences of 18 to 30 nucleotides that distinguished methanotrophs at the family or genus level. Candidate oligonucleotide sequences were then examined for specificity using the basic BLAST search and Probe Match functions of GenBank and the RDP-II, respectively (5, 42). Except as described below, only sequences exhibiting high specificity for methanotrophs and retrieving a majority of the sequences in their target groups were pursued further.

Tα determination and specificity testing. Oligodeoxynucleotides were synthesized commercially (DNAgeny, Malvern, Pa.). Each probe was characterized by empirical determination of its midpoint dissociation temperature (Tα) using a serial washing procedure with progressively higher temperatures in a PCR thermal cycler as described by Gulledge and Cavanaugh (32). All Tα curves were determined using triplicate plates for both positive and negative controls (see Fig. 1). The ability of each probe to distinguish between positive and negative controls was screened in Northern dot blot hybridization assays, as described below, using total RNA from reference cultures representing target strains as positive controls and total RNA from reference cultures representing nontarget strains with 1- or 2-base mismatches as negative controls. In all but two cases, a strain with a single-base mismatch with the probe was used as a negative control (Table 1). Because no nontarget organisms that had fewer than two mismatches with probe Am454 were identified, an organism with two mismatches was used as a negative control. Also, because no potential control organisms with fewer than four mismatches to probe Mcd77 were identified, we designed a probe with a single mismatch at position 15 to serve as a negative control (Table 1).

RNA extraction from bacterial cultures. Pure cultures were grown to late-log phase in 40 ml of liquid growth medium and centrifuged at 5,000 × g for 10 min at 4°C. Total RNA was extracted selectively from cell pellets using the FastPrep bead beater system with the FastRNA Blue kit according to the manufacturer’s protocol (Bio 101, La Jolla, Calif.). Cells were beaten in the FP120 bead beater system with a speed of 6 m/s. After extraction and centrifugation, the RNA pellets were air dried, resuspended in diethyl pyrocarbonate-treated H2O, and stored at −80°C.

RNA dot blotting and hybridization. Northern dot blots were prepared from RNA extracts as described previously (48) using a MiniTowel I Microsample Filtration Manifold (Schleicher & Schuell, Keene, N.H.). Blots were prepared with 100 ng of 16S rRNA per dot to be blotted, assuming that 16S rRNA represented 27% of total RNA (47), as described previously (49).

Oligonucleotide probes were labeled enzymatically with 32P (49), and hybridization assays were carried out as described previously (48). Labeled oligonucleotides were hybridized to the dot blots overnight at 30°C, finishing with two 30-min rinses at the appropriate Tα for each probe (Table 1). Oligonucleotide labeling of the dot blots was analyzed by radiodensitometry using a BAS-MS 2025 imaging plate and a Fuji 2000 PhosphorImager, with MacBas, version 2.5, image analysis software (Fuji Medical Systems, Stamford, Conn.).

Nucleotide sequence accession numbers. The new sequences of the 16S rRNA genes of Methylosphaerina S1 NCIMB 11130, Methylibacterium luteus NCIMB 11914, Methylosphaerina S1 NCIMB 11130, and Methylibacterium marinus strain A45 have been deposited in GenBank (accession numbers AF304194 to AF304197).

RESULTS AND DISCUSSION

Overview. In recent years, interest in the physiology, ecology, and evolution of methanotrophs has intensified, and there is high demand for tools to facilitate quantitative studies of in situ methanotroph community structure (21, 34, 46, 50). Our objectives were to develop phylogenetic oligonucleotide probes for analysis of methanotrophs at the family and genus levels and to optimize the probes for use in quantitative hybridization through empirical determination of their Tα under standard hybridization conditions.

Visual comparison of aligned 16S rRNA reference sequences initially revealed 36 potential probe sequences for fur-
TABLE 1. Oligonucleotide probes targeting methanotrophic bacteria

<table>
<thead>
<tr>
<th>Target group(s)</th>
<th>Reference strain</th>
<th>Positive control(s)</th>
<th>Negative controls</th>
<th>Oligonucleotide probe(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylocella palustris</td>
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<td>Methylocella palustris</td>
<td>Methylophilic methanotrophs</td>
<td>Mtr, Methylomonas, Methylocaldum, Methylococcus, Methylobacter, Mlm, Methylomicrobium, Mcd</td>
</tr>
<tr>
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<td>Streptomyces albus</td>
<td>Methylocaldum gracile</td>
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</tr>
<tr>
<td>Methylocystis strain K8</td>
<td>M. Acidiphilum methanotroph</td>
<td>Methylocystis strain K8</td>
<td>Methylocaldum gracile</td>
<td>Mtr, Methylomonas, Methylococcus, Methylobacter, Mlm, Methylomicrobium, Mcd</td>
</tr>
<tr>
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</tr>
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</table>

**Notes:**

- Boldfaced letters in probe sequences correspond to positions of mismatch in the corresponding negative controls.
- See Fig. 2 and 3 for detailed coverage information.
- Previously published as PCR primer Mb1007r (44).
- Because no negative-control organism with one or two mismatches was available, probe Mcd77 was modified by base (A to T at position 15) and hybridized to Methylocaldum gracile, Methylophilic methanotrophs only as a group.

**FIG. 1.** Typical $T_d$ curves illustrating the ability of the probes to discriminate quantitatively between target and nontarget rRNA with a 1- or 2-base mismatch.

**Probes:**

- **Mmb482:** Methylocaldum gracile
- **Mmb1007:** Methylomonas rubra
- **Mmb1007r:** Methylomonas album
- **Mcd77:** Methylocaldum gracile

**Probes for α-methanotrophs:** Two family-level probes, Am445 and Am976, perfectly match the 16S rRNA sequences of nearly all known α-methanotrophs (Table 1), including some novel strains recently isolated from landfill soils (59) and lake sediments (22). *Methyloloccya palustris* strain K8, a novel acidophilic methanotroph isolated recently from a northern peat bog and the only cultured representative of its genus (24), was the only α-methanotroph whose 16S rRNA sequence was not covered by either probe. Because these probes do not distinguish between the *Methylosinus* and *Methylocystis* genera, they can detect α-methanotrophs only as a group. No oligonucleotide signatures that distinguish between these two genera were identified.

**Probes for γ-methanotrophs:** For γ-methanotrophs, we identified both family- and genus-level probes. Together,
Two family-level probes (Gm633 and Gm705) covered 82% of index of overall sequence quality. Under "within the entire sequence that indicate ambiguous bases, shown as an 
sequence available in probe target region. 
Union of Pure and Applied Chemistry [IUPAC] ambiguity code for C 
probe target region that are consistent with the probe sequence. For 
based on criteria outlined in Materials and Methods. An open dia-
on a solid background, the apparent mismatches were disregarded 
corresponding 16S rRNA sequence. Where a number is shown in white 
dicates identity between the probe and a nonmethanotroph; numbers 
identity between the probe and a target sequence; cross-hatching in-
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the 16S rRNA molecule. If the two apparent insertions are 
indicated base change would violate the secondary structure of 
family-level detection of the closely 
related \( \gamma \)-methanotroph genera and the methanotrophic endosymbionts of marine mollusks. Gm633 was more limited, but it 
six 
Methylomonas spp., but all representatives of the gen-
us were covered only when the three Mlm probes were combined. 
Three probes covered all representatives of the two recog-
nized thermophilic genera, Methylcococcus and Methylocaldum. 
Mlc123 and Mlc1436 each matched all Methylcococcus strains 
now sequences were available. PCR primers corresponding to these two 
probes might be ideal for specific amplification of nearly complete 
(\( \sim 1,300 \)-bp) 16S rRNA genes from Methylcococcus strains 
environmental samples. Probe Mcd77 covered the three 
recognized strains of the recently described genus Methylocaldum. 
The target region was unique, and a Probe Match analysis 
retrieval no sequences with fewer than four mismatches 
from Non-Methylocaldum species. 
The complete suite of \( \gamma \)-methanotroph probes covered 97% of 
the strains listed in Fig. 3; only two sequences were not 
covered. One is that of Methylomonas methanica strain 81Z, 
cultures of which are no longer extant and whose affiliation 
with the genus Methylomonas was never verified (J. P. Bowman and P. N. Green, personal communication). Because this 
sequence is of low overall quality (3.3% ambiguity), one or more 
of the indicated mismatches could be incorrect. The other or-
ganism not covered by the probes is a novel thermophilic meth-
antroph, “Methylothermus” sp. strain HB. Because it is the 
only known \( \gamma \)-methanotroph that is polyphyletic with respect to 
the family Methylcococcaceae (8), this result was expected. 

**FIG. 2.** Range of strain coverage for oligonucleotide probes target-
ing \( \alpha \)-methanotrophs (Am). % Ambiguity, percentage of positions within 
the entire sequence that indicate ambiguous bases, shown as an 
index of overall sequence quality. Under ‘Probes,’ solid fill indicates 
identity between the probe and a target sequence; cross-hatching in-
dicates identity between the probe and a nonmethanotroph; numbers 
of mismatches between the probe sequence and the 
16S rRNA sequence for 
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the family Methylcococcaceae (8), this result was expected. 

**Probe specificity and optimization for quantitative hybrid-
ization.** The probes described here are intended to quantify 
16S rRNA from specific microbial populations against a back-
ground of many unknown populations in environmental sam-
ple. The probes must discriminate against unknown, non-target 
16S rRNA that may have a difference of only 1 base from 
the intended target. The primary factor for achieving stringent 
specificity and quantitative hybridization of 16S rRNA from 
environmental samples is accurate determination of the melting 
characteristics of the probe-target duplex. Hence, empirical 
determination of the \( T_m \) is essential (32, 54). We have optimi-
zed the probes presented here for stringent discrimination against non-target RNA and also for quantitative hybridization 
by empirically determining the \( T_m \) for each probe. 

The \( T_m \)'s of the probes ranged from 49 to 57°C (Table 1). 
When Northern blots were hybridized overnight and then washed 
at the appropriate \( T_m \), target and non-target rRNAs were visu-
ally distinguishable on blots and yielded quantitatively distinct 
results when analyzed using a scintillation counter (Fig. 1) or a 
phosphorimag (data not shown). These results verify that the 
use of known concentrations of reference rRNA as standards 
will permit quantitative analysis of environmental RNA pos-
sessing the target sequence, as demonstrated previously (49, 54).
Probes Am445, Mmb1007, Mlm482, Mlm732b, Mlc123, and Mcd77 each exhibited at least two base mismatches against any nonmethanotroph sequence, whereas probes Gm633, Gm705, Mlb482, Mmb482, Mlm732a, and Mlc1436 each exhibited at least one base mismatch with any nonmethanotroph sequence. Probes Mlb662, Mmb482, and Mmb1007 matched sequences from one to four $\gamma$-methanotrophs outside their respective target genera (Fig. 3). Although we consider this problem to be minor, these probes could yield ambiguous results for fine-scale descriptions of $\gamma$-$\delta$-methanotroph communities. All other genus-level probes were specific to their intended target genera. The $\alpha$- and $\gamma$-methanotroph probes had no cross-family hybridization potential.

Two probes, Am976 and Mlb662, present the more serious problem of complementing 16S rRNA sequences of some non-methanotrophic bacteria. They have been retained despite this weakness for two reasons. First, they are needed to ensure complete coverage of their target groups, in combination with other probes, when broad-spectrum probing is desired. Second, they were deemed particularly useful for certain experi-

FIG. 3. Range of strain coverage for oligonucleotide probes targeting $\gamma$-methanotrophs (Gm). Mlc, Methylococcus; Mcd, Methylocaldum. % Ambiguity, percentage of positions within the entire sequence that indicate ambiguous bases, shown as an index of overall sequence quality. The unpublished 16S rRNA sequence for *Methylocaldum methanica* strain S1 (marked with a star in the “GenBank accession number” column) is available as RDP sequence Mlm.metha1 (C. R. Woese, 1991). Under “Probes,” solid fill indicates identity between the probe and a target sequence; diagonal hatching indicates identity between the probe and a nontarget $\gamma$-$\delta$-methanotroph strain; cross-hatching indicates identity between the probe and a nonmethanotroph; numbers are numbers of mismatches between the probe sequence and the corresponding 16S rRNA sequence. Where a number is shown in white on a solid background, the apparent mismatches were disregarded based on criteria outlined in Materials and Methods. An open diamond denotes the occurrence of one or more ambiguous bases in the probe target region that are consistent with the probe sequence. For example, if the probe has an A corresponding to a Y (IUPAC ambiguity code for C or T), then the possible T is consistent with the probe sequence. ns, no sequence available in probe target region.

Probes Am445, Mmb1007, Mlm482, Mlm732b, Mlc123, and Mcd77 each exhibited at least two base mismatches against any nonmethanotroph sequence, whereas probes Gm633, Gm705, Mlb482, Mmb482, Mlm732a, and Mlc1436 each exhibited at least one base mismatch with any nonmethanotroph sequence. Probes Mlb662, Mmb482, and Mmb1007 matched sequences from one to four $\gamma$-methanotrophs outside their respective target genera (Fig. 3). Although we consider this problem to be minor, these probes could yield ambiguous results for fine-scale descriptions of $\gamma$-methanotroph communities. All other genus-level probes were specific to their intended target genera. The $\alpha$- and $\gamma$-methanotroph probes had no cross-family hybridization potential.

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mental approaches, such as monitoring of CH₄ enrichment cultures, use as PCR primers in cases where amplification approaches, such as monitoring of CH₄ enrichment products are to be sequenced for identification, or analysis of community composition in environmental samples where the non-target organisms with which the probes hybridize should be minor components of the community. For instance, because marine Cycloclastics spp. were the only nonmethanotrophs that matched Mlb662 (Fig. 3), this probe might be appropriate for probing nonmarine samples.

**Probing the database.** The GenBank database contains thousands of bacterial 16S rRNA gene sequences from cultures and environmental clones (6). Hence, “probing” this database should provide a powerful assessment of a probe’s ability to select specifically for methanotroph sequences against a background of myriad nonmethanotroph sequences. We subjected each probe sequence to a basic BLAST search (5) and examined sequences retrieved with an identical match. Only sequences identified as 16S rRNA genes were considered. The organism identifications were based solely on information provided in the accession records or in publications cited therein.

Eleven of 14 probes retrieved only sequences that were identified as methanotrophs (Table 2). Probes Am976, Mlb662, and Mlc1436 retrieved a number of sequences representing many of the community. For instance, because marine Cycloclastics spp. were the only nonmethanotrophs that matched Mlb662 (Fig. 3), this probe might be appropriate for probing nonmarine samples.

**Summary and conclusions.** The breadth and specificity of the probes reported here are unprecedented, providing 97% coverage of the 87 methanotroph 16S rRNA sequences examined (Fig. 2 and 3). Several new methanotroph genera that have been proposed recently following the isolation of novel strains are covered. Of the three strains apparently not covered by the probes, one is no longer extant and the available 16S rRNA sequence is of low overall quality, bringing into question whether the indicated probe mismatches are correct. The other two strains (Methyllobacteria palustris str. K and Methylotrophussp. strain HB) that did not match any probe are polyphyletic with respect to the Methyllobacteria and Methylotrophus, thus reflecting the high specificity of the probes to the phylogenetic clades they were designed to target. Initial results from studies with several soils indicate that the probes are effective for studying methanotroph communities in soil (unpublished data), perhaps the most difficult substrate on which to perform quantitative hybridization assays (4). Hence, all of the methanotroph taxa that have become well known through years of laboratory studies, as well as several recently described taxa, can now be studied at both the family and genus levels in environmental samples by using the probes reported here.

**ACKNOWLEDGMENTS**

J. Gulledge and A. Ahmad contributed equally to this work.

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**REFERENCES**


