

## Design and Evaluation of Useful Bacterium-Specific PCR Primers That Amplify Genes Coding for Bacterial 16S rRNA

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Received 8 August 1997/Accepted 14 November 1997

**We report the design and evaluation of PCR primers 63f and 1387r for amplification of 16S rRNA genes from bacteria. Their specificity and efficacy were tested systematically with a variety of bacterial species and environmental samples. They were found to be more useful for 16S rRNA gene amplification in ecological and systematic studies than PCR amplimers that are currently more generally used.**

Until recently, the estimation of bacterial biodiversity has been hampered by limitations associated with cultivating bacteria from natural environments. An uncultured fraction has been recognized to be a major component of all microbial communities (7). The application of molecular approaches to the characterization of bacterial communities has overcome the requirement for prior cultivation of community members. In particular, the analysis of 16S rRNA genes, aided by using PCR to amplify target sequences in environmental samples, has enabled molecular ecologists to provide better estimates of bacterial diversity (1). PCR primers for amplification of 16S rRNA genes are widely available (6, 13). However, a problem with several commonly used amplimers is that they have been constructed theoretically, using the (incomplete) database of 16S rRNA sequences from cultured organisms, and have not been tested systematically. Hence, empirical testing is essential to confirm PCR primer specificity prior to their use in PCRs with environmental samples.

Primers for PCR described by Lane (6), which have been used by many other workers (5, 8), consistently failed to work with some difficult samples generated in our work. DNA samples extracted from various sources, including deep sea sediment, oral bacteria, and bacteria isolated from epilithon (biofilms associated with stones in lotic habitats), were found to be poor templates for amplification of the 16S rRNA gene with amplimers such as 27f and either 1492r or 1392r (numbering is based on the *Escherichia coli* 16S rRNA gene [3]). We had attempted to optimize the PCR conditions for 27f-1392r by altering the annealing temperature, Mg<sup>2+</sup> concentration, and DNA template concentration and also by including the PCR additives bovine serum albumin, Triton X-100, T4 gene 32 protein, polyethylene glycol 8000 and glycerol. Finally, we decided to redesign the amplimers, and when we used 63f and 1387r with the difficult DNA samples, we were successful.

The wide adoption of amplimers 27f, 1392r, and 1492r is empirically based, and although their utility for investigating

the molecular ecology of natural bacterial communities is often assumed, to our knowledge they have not been systematically tested. In this communication, we describe a new set of amplimers which were designed to be universal for the domain *Bacteria* (14) and their testing on a range of pure cultures and difficult natural samples.

Unless otherwise noted, genomic DNA was extracted from pure cultures, reference strains, and environmental samples by a modification of the method of Ausubel et al. (2). DNA from marine sediment samples (1 to 2 g), collected as described previously (9), was extracted by a modification of the method of Rochelle et al. (10). After the lysozyme step, 100  $\mu$ l of proteinase K (18 mg/ml; Sigma) was added, and the solution was further incubated for 1 h at 37°C. Second, the phenol-chloroform step was replaced by adding one-half volume of 7.5 M ammonium acetate, and the mixture was centrifuged at  $11,220 \times g_{av}$  for 20 min at 4°C. DNA from the three *Leptospira* strains was obtained as a thermolysate (2a).

Two PCR primers were designed (Oligo, version 3.4; National Biosciences Inc.) to amplify approximately 1,300 bp of a consensus 16S rRNA gene (6): forward primer 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387r (5'-GGG CGG WGT GTA CAA GGC-3') (Pharmacia). Primers 27f and 1392r (6) were also used.

The PCR mixtures (100  $\mu$ l) contained 20 pmol of each appropriate primer, 200  $\mu$ M each deoxynucleoside triphosphate, *Taq* extender PCR buffer (Stratagene Ltd.), 0.5 U of *Taq* extender (Stratagene), and 0.5 U of *Taq* polymerase (Boehringer). Approximately 200 to 300 ng of DNA from a test strain culture and subnanogram quantities of sediment DNA were added to PCRs. In addition, 2  $\mu$ g of T4 gene 32 protein (Pharmacia) was included in PCRs of sediment DNA. PCR was performed with one of the following thermal cyclers: Hybaid Omnigene, Omni-E, or TR1; Perkin-Elmer 460; or MJ Research PTC-100. All cyclers were programmed to perform 30 cycles consisting of 95°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min followed by a final extension step of 5 min at 72°C. PCR products were visualized by agarose gel electrophoresis (11).

The specificities of the new primers were tested in PCRs

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TABLE 1. Species of bacteria and archaea tested in PCRs using amplicon 63f and 1387f to amplify 16S rRNA genes

| Strain <sup>a</sup>                                                         | Woese grouping                                          | PCR amplification <sup>b</sup> |
|-----------------------------------------------------------------------------|---------------------------------------------------------|--------------------------------|
| <i>Chlamydia psittaci</i> <sup>c</sup>                                      | <i>Planctomyces</i> and relatives                       | +                              |
| <i>Synechocystis</i> sp. strain PCC 6803                                    | Cyanobacteria and chloroplasts                          | +                              |
| <i>Synechococcus</i> sp. strain WH 7803                                     | Cyanobacteria and chloroplasts                          | +                              |
| <i>Synechococcus</i> sp. strain PCC 7942                                    | Cyanobacteria and chloroplasts                          | +                              |
| <i>Nostoc</i> sp.                                                           | Cyanobacteria and chloroplasts                          | +                              |
| <i>Anabaena</i> sp. strain PCC 7120 <sup>f</sup>                            | Cyanobacteria and chloroplasts                          | +                              |
| <i>Runella</i> sp.                                                          | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Prevotella oris</i>                                                      | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Prevotella veroralis</i>                                                 | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Prevotella buccalis</i>                                                  | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Prevotella intermedia</i>                                                | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Cytophaga ochracea</i>                                                   | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | +                              |
| <i>Rhodobacter sphaeroides</i>                                              | α-Proteobacteria                                        | +                              |
| <i>Alcaligenes eutrophus</i> JMP222                                         | β-Proteobacteria                                        | +                              |
| <i>Myxococcus xanthus</i> NCIMB 9412 <sup>e</sup>                           | δ-Proteobacteria                                        | +                              |
| <i>Desulfovibrio desulfuricans</i> <sup>c</sup>                             | δ-Proteobacteria                                        | +                              |
| <i>Desulfovibrio salexigens</i> <sup>c</sup>                                | δ-Proteobacteria                                        | +                              |
| <i>Desulfovibrio sapovorans</i>                                             | δ-Proteobacteria                                        | +                              |
| <i>Desulfovibrio postgatei</i>                                              | δ-Proteobacteria                                        | +                              |
| <i>Desulfovibrio propionicus</i> <sup>c</sup>                               | δ-Proteobacteria                                        | +                              |
| <i>Wolinella succinogenes</i> ATCC 29543                                    | ε-Proteobacteria                                        | +                              |
| <i>Fusobacterium nucleatum</i> <sup>f</sup>                                 | ε-Proteobacteria                                        | +                              |
| <i>Campylobacter concisus</i> ATCC 33237                                    | ε-Proteobacteria                                        | +                              |
| <i>Campylobacter sputorum</i> subsp. <i>sputorum</i> ATCC 35980             | ε-Proteobacteria                                        | +                              |
| <i>Campylobacter rectus</i> ATCC 33238                                      | ε-Proteobacteria                                        | +                              |
| <i>Campylobacter curvus</i> ATCC 35224                                      | ε-Proteobacteria                                        | +                              |
| <i>Campylobacter showae</i> ATCC 51146                                      | ε-Proteobacteria                                        | +                              |
| <i>Escherichia coli</i> HB101 <sup>c</sup>                                  | γ-Proteobacteria                                        | +                              |
| <i>Acinetobacter calcoaceticus</i> AC8250                                   | γ-Proteobacteria                                        | +                              |
| <i>Pseudomonas putida</i> UWCI <sup>c</sup>                                 | γ-Proteobacteria                                        | +                              |
| <i>Klebsiella pneumoniae</i>                                                | γ-Proteobacteria                                        | +                              |
| <i>Eubacterium nodatum</i> ATCC 33099 <sup>e</sup>                          | Clostridia and relatives                                | +                              |
| <i>Eubacterium brachy</i> ATCC 33089                                        | Clostridia and relatives                                | +                              |
| <i>Eubacterium timidum</i> ATCC 33092, ATCC 33093                           | Clostridia and relatives                                | +                              |
| <i>Eubacterium tardum</i> SC68, <sup>e</sup> SC88P                          | Clostridia and relatives                                | +                              |
| <i>Eubacterium exiguum</i> ATCC 700122                                      | Clostridia and relatives                                | +                              |
| <i>Eubacterium minutum</i> ATCC 700079, ATCC 700080                         | Clostridia and relatives                                | +                              |
| <i>Eubacterium saburreum</i> ATCC 33271, ATCC 33318, ATCC 33319, ATCC 43850 | Clostridia and relatives                                | +                              |
| <i>Pseudoramibacter alactolyticus</i> DSM 3980 (= ATCC 23263)               | Clostridia and relatives                                | +                              |
| <i>Ruminococcus torques</i> ATCC 27756                                      | Clostridia and relatives                                | +                              |
| <i>Veillonella atypica</i> ATCC 17744                                       | Clostridia and relatives                                | +                              |
| <i>Veillonella dispar</i> ATCC 17748                                        | Clostridia and relatives                                | +                              |
| <i>Veillonella parvula</i> ATCC 10790                                       | Clostridia and relatives                                | +                              |
| <i>Veillonella caviae</i> ATCC 33540                                        | Clostridia and relatives                                | +                              |
| <i>Veillonella criceti</i> ATCC 17747                                       | Clostridia and relatives                                | +                              |
| <i>Veillonella rodentium</i> ATCC 17743                                     | Clostridia and relatives                                | +                              |
| <i>Veillonella ratti</i> ATCC 17746                                         | Clostridia and relatives                                | +                              |
| <i>Eubacterium lentum</i> NCTC 11813, <sup>e</sup> ATCC 25559               | Gram positive, high G+C                                 | +                              |
| <i>Micrococcus luteus</i>                                                   | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces naeslundii</i> genosp. 1 ATCC 12104                          | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces naeslundii</i> genosp. 2 WVU 627                             | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces israelii</i> ATCC 12102, ATCC 10048, MTU 01003, MTU 01004    | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces gerencseriae</i> ATCC 23860                                  | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces odontolyticus</i> ATCC 17929                                 | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces meyeri</i> ATCC 35568                                        | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces georgiae</i> ATCC 49285                                      | Gram positive, high G+C                                 | +                              |
| <i>Actinomyces viscosus</i> ATCC 15987                                      | Gram positive, high G+C                                 | +                              |
| <i>Propionibacterium acnes</i> ATCC 11827                                   | Gram positive, high G+C                                 | +                              |
| <i>Bacillus cereus</i> NCTC 7464                                            | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Bacillus popilliae</i> NRRL B2309M <sup>c</sup>                          | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Peptostreptococcus anaerobius</i>                                        | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i> ATCC 9649        | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Lactobacillus coryniformis</i> subsp. <i>torquens</i> ATCC 25600         | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Streptococcus salivarius</i> ATCC 7073                                   | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Streptococcus sanguis</i> ATCC 10556                                     | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | +                              |
| <i>Eubacterium saphenum</i> ATCC 49989                                      | Gram positive, low G+C                                  | +                              |
| <i>Leptospira inadai</i> ATCC 43289                                         | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | + <sup>d</sup>                 |

Continued on following page

TABLE 1—Continued

| Strain <sup>a</sup>                                                | Woese grouping                                    | PCR amplification <sup>b</sup> |
|--------------------------------------------------------------------|---------------------------------------------------|--------------------------------|
| <i>Leptospira kirschneri</i> ATCC 23469                            | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | + <sup>d</sup>                 |
| <i>Leptospira wolbachii</i> ATCC 43284                             | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | + <sup>d</sup>                 |
| <i>Treponema denticola</i> ATCC 35405, ATCC 33520                  | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Treponema vincentii</i> ATCC 35580                              | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Treponema pallidum</i> subsp. <i>pallidum</i> Nichols strain    | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Treponema phagedenis</i> biovar Kazan ATCC 27087, biovar Reiter | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Treponema socranskii</i> subsp. <i>socranskii</i> ATCC 35536    | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Treponema medium</i> G7201                                      | <i>Spirochaeta-Treponema-Borrelia</i> subdivision | +                              |
| <i>Methanosarcina frisia</i>                                       | <i>Euryarchaeota, Methanosarcinales</i>           | –                              |
| <i>Halobacterium halobium</i> NCMB 2090                            | <i>Euryarchaeota, extreme halophile</i>           | –                              |

<sup>a</sup> Strains not identified as originating from specific culture collections were well-characterized strains from culture collections held by participating laboratories.

<sup>b</sup> +, positive reaction, i.e., a single amplicon of the correct predicted size (ca. 1,300 bp); –, no PCR product.

<sup>c</sup> The PCR product of this strain was sequenced to ensure that it represented the 16S rRNA gene from the organism.

<sup>d</sup> The 16S rRNA gene from this *Leptospira* species was successfully amplified; however, the resulting amount was approximately 90% less than the signal generated from an amplification of the 16S rRNA gene of *E. coli*.

with template DNA from cultures of well-characterized species representing the major groups of the domain *Bacteria* (Table 1) (14). Since DNA from the *Thermotogales*, green non-sulfur bacteria, and *Fibrobacteria* groups was not available for testing, the sequence similarities between amplimers 63f and 1387r and the 16S rRNA genes of selected species from those groups were assessed by aligning the amplimer sequences with the appropriate prealigned sequences obtained from the Antwerp rRNA database (12) (Table 2). In the comparison of alignments shown in Tables 2 and 3, one main point emerged. Amplimers 63f and 1387r successfully amplified 16S rRNA genes from species showing higher levels of theoretical 5' mismatches than amplimer pair 27f-1392r, in some cases with 3' mismatches in 1387r.

In a range of experimental studies carried out in the participating laboratories, primers 63f and 1387r were used successfully and consistently to amplify 16S rRNA genes from template DNA extracted from a variety of organisms: organisms identified as belonging to the coryneform and *Micrococcus* genera (gram-positive, high-G+C bacteria), cultured for the first time from concrete; *Eubacterium* species cultured from dental abscesses; novel  $\delta$ -proteobacteria (sulfate- and iron-reducing bacteria); epilithic samples; and deep sea sediments. Conversely, amplimer pair 27f-1392r failed to amplify the 16S rRNA genes of many of these test samples.

Our results provide no clear theoretical explanation for why

amplimer pair 63f-1387r was so much more successful than 27f-1392r. One suggestion is that the latter amplimers are not optimal for PCR since 27f may form an intramolecular duplex with a 5' overhang and may thus be susceptible to the 5'→3' exonuclease activity of *Taq* polymerase. Any resultant removal of 5' nucleotides from 27f (possibly six in total) would affect the annealing temperature of the primer pair ( $\Delta T_m$  of 13.6°C instead of 1.6°C) and also result in unfavorable intermolecular complementarity between 27f and 1392r, leading to binding of the 3' ends. An alternative explanation comes from a recent computer analysis of the potential of primers to hybridize with 16S rRNA genes (4). In this study, a primer designed for the conserved area of the 16S rRNA gene, which was also used for 63f, was found to have a greater hybridization potential than the conserved area used for 27f.

In conclusion, although 63f and 1387r showed some theoretical bias, in practice they were more successful than amplimer pair 27f-1392r and amplified 16S rRNA genes from a wider range of bacteria than other primers which are commonly used for bacterial community analysis. So far as we are aware, the other primers have not been tested in the systematic way described for 63f-1387r in this paper. The results presented here suggest that the latter primer pair may be better suited for this type of molecular ecological analysis, the aim of which is to minimize PCR bias, and underline the point that the theoretical design of PCR amplimers is only the beginning

TABLE 2. Theoretical alignment of sequences of amplimers 63f and 1387r with database sequences of 16S rRNA genes from species not tested by PCR

| Strain                           | Woese grouping                          | Sequence vs <sup>a</sup> :              |                            |
|----------------------------------|-----------------------------------------|-----------------------------------------|----------------------------|
|                                  |                                         | 63f                                     | 1387r                      |
| <i>Fibrobacter intestinales</i>  | <i>Fibrobacter</i> spp.                 | <b>CAGG</b> <u>CCTAACACATGCA</u> –AGTC  | <b>CGGA</b> ACATGTGWGGCGGG |
| <i>Chlorobium limicola</i>       | Green non-sulfur                        | <b>AGT</b> <u>GCTTATACATGCA</u> –AGTC   | GCCTTGACACACCGCCC          |
| <i>Chloroflexus aurantiacus</i>  | Green non-sulfur                        | <b>CGT</b> <u>GCCTAACACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Herpetosiphon aurantiacus</i> | Green non-sulfur                        | <b>CGT</b> <u>GCCTAATGCATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Thermomicrobium roseum</i>    | Green non-sulfur                        | <b>CGT</b> <u>GCCTAATGCATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Deinococcus radiodurans</i>   | Radioresistant micrococci and relatives | <b>CGT</b> <u>GCTTAAGACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Thermus aquaticus</i>         | Radioresistant micrococci and relatives | <b>CGT</b> <u>GCCTAAGACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Thermus ruber</i>             | Radioresistant micrococci and relatives | <b>TAT</b> <u>GCCTAAGACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Fervidobacterium nodosum</i>  | <i>Thermotogales</i>                    | <b>CGT</b> <u>GCCTAACACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |
| <i>Thermotoga elfii</i>          | <i>Thermotogales</i>                    | <b>CGT</b> <u>GCTTAAACACATGCA</u> –AGTC | <b>GCT</b> TTGACACACCGCCC  |
| <i>Thermotoga maritima</i>       | <i>Thermotogales</i>                    | <b>CGT</b> <u>GCCTAACACATGCA</u> –AGTC  | GCCTTGACACACCGCCC          |

<sup>a</sup> Nucleotides underlined and in boldface differ from corresponding nucleotides in amplimer.

TABLE 3. Alignment of sequences of amplimers 63f-1387r and 27f-1392r with the corresponding regions of 16S rDNA genes of representative species used in PCR

| Strain                                                                    | Woese group                                             | Sequence vs primers <sup>a</sup> :                                |                                                                   |                                                                             |
|---------------------------------------------------------------------------|---------------------------------------------------------|-------------------------------------------------------------------|-------------------------------------------------------------------|-----------------------------------------------------------------------------|
|                                                                           |                                                         | 27f and 63f                                                       |                                                                   | 1387r and 1392r                                                             |
|                                                                           |                                                         | <b>AGAGTTTGATCMTGGCTC<sup>b</sup></b>                             | <b>CAGGCCTAACACATGCAA-GTC<sup>c</sup></b>                         | <b>CRTGTGTGGCGGGCA<sup>d</sup></b><br><b>CGGAACATGTGMGGCGGG<sup>e</sup></b> |
| <i>Rhodobacter sphaeroides</i> ATH 2.4.1                                  | α-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCAGGCCTAACACATGCAA-GTC        | GAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Alcaligenes eutrophus</i>                                              | β-Proteobacteria                                        | AAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGTGGCGTGCCTAAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Campylobacter concisus</i> (FDC 288)                                   | ε-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGTGGCGTGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACTCACCGCCCGT                                                        |
| <i>Campylobacter curvus</i> (ATCC 35224 <sup>f</sup> ; VPI 9584)          | ε-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACTCACNGCCCGT                                                        |
| <i>Campylobacter rectus</i> (CCUG 19168)                                  | ε-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACTCACCGCCCGT                                                        |
| <i>Campylobacter showae</i> (CCUG 3054)                                   | ε-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAGTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACTCACCGCCCGT                                                        |
| <i>Desulfobacter postgatei</i>                                            | δ-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GNNTTGTACACACCGCCNGT                                                        |
| <i>Desulfovibrio desulfuricans</i> (ATCC 27774)                           | δ-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Myxococcus xanthus</i> DK1622 (ATCC null)                              | δ-Proteobacteria                                        | GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Acinetobacter calcoaceticus</i>                                        | γ-Proteobacteria                                        | AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTTAACACATGCAA-GTC       | AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Escherichia coli</i>                                                   | γ-Proteobacteria                                        | AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTTAACACATGCAA-GTC       | AAGAGTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTTAACACATGCAA-GTC       | CCTTGTACACACCGCCCGT                                                         |
| <i>Pseudoramibacter alactolyticus</i> DSM 3980, ATCC 23263                | γ-Proteobacteria                                        | -AGAGTTTGATCMTGGCTCAGGACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | -AGAGTTTGATCMTGGCTCAGGACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Bacillus cereus</i> F4810/72 serotype 1 (NCTC 11143)                   | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Bacillus popilliae</i>                                                 | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAGACATGCAA-GTC        | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Lactobacillus coryniformis</i>                                         | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i> strain Calvert | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Streptococcus salivarius</i> C699 (ATCC 13419)                         | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTA       | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GCNTNGTACACACCGCCCGT                                                        |
| <i>Streptococcus sanguis</i> (NCTC 7863; ATCC 15300)                      | <i>Bacillus-Lactobacillus-Streptococcus</i> subdivision | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTN       | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTAAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Eubacterium angustum</i>                                               | Clostridia and relatives                                | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Eubacterium fossor</i>                                                 | Clostridia and relatives                                | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCNTTGTACACACCGCCCGT                                                        |
| <i>Eubacterium lentum</i>                                                 | Clostridia and relatives                                | -AGAGTTTGATNNTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | -AGAGTTTGATNNTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Eubacterium limosum</i>                                                | Clostridia and relatives                                | GAGAGTTTGATCCTGGCTCAGGACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Eubacterium xylanophilum</i>                                           | Clostridia and relatives                                | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Nostoc muscorum</i>                                                    | Cyanobacteria and chloroplasts                          | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Prevotella buccalis</i>                                                | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | TAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | TAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Prevotella intermedia</i>                                              | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Prevotella oris</i>                                                    | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Prevotella veroralis</i>                                               | <i>Flexibacter-Cytophaga-Bacteroides</i> phylum         | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGATNAACGCTAGCTACAGGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Actinomyces viscosus</i>                                               | Gram positive, high G+C                                 | GAGAGTTNATCCTGGCTCAGGACGAANGCTNGCGGCATGCCTTAACACATGCAA-GTC        | GAGAGTTNATCCTGGCTCAGGACGAANGCTNGCGGCATGCCTTAACACATGCAA-GTC        | GGCTTGTACACACCGCCCGT                                                        |
| <i>Propionibacterium acnes</i>                                            | Gram positive, high G+C                                 | GAGAGTTTGATCCTGGCTCAGGACGAANGCTNGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGGACGAANGCTNGCGGCATGCCTTAACACATGCAA-GTC       | GTCTTGTACACACCGCCCGT                                                        |
| <i>Chlamydia psittaci</i>                                                 | <i>Planctomyces</i> and relatives                       | GAGAATTTTGATCCTGGTTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC      | GAGAATTTTGATCCTGGTTCAGATTGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC      | GCCTTGTACACACCGCCCGT                                                        |
| <i>Leptospira inadai</i> ATCC 43289                                       | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | ?????????????????????????????STARCGTTGGCGGCATGCCTTAACACATGCAA-GTC | ?????????????????????????????STARCGTTGGCGGCATGCCTTAACACATGCAA-GTC | ACCTTGTACACACCGCCCGT                                                        |
| <i>Leptospira kirschneri</i> ATCC 23469                                   | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | ?????????????????????????????STARCGCTGGCGGCATGCCTTAACACATGCAA-GTC | ?????????????????????????????STARCGCTGGCGGCATGCCTTAACACATGCAA-GTC | ACCTTGTACACACCGCCCGT                                                        |
| <i>Leptospira wolbachii</i> ATCC 43284                                    | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | ?????????????????????????????STARCGCTGGCGGCATGCCTTAACACATGCAA-GTC | ?????????????????????????????STARCGCTGGCGGCATGCCTTAACACATGCAA-GTC | ACCTTGTACACACCGCCCGT                                                        |
| <i>Treponema denticola</i>                                                | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |
| <i>Treponema phagedenis</i>                                               | <i>Spirochaeta-Treponema-Borrelia</i> subdivision       | GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GAGAGTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTTAACACATGCAA-GTC       | GCCTTGTACACACCGCCCGT                                                        |

<sup>a</sup> Primer sequences are written 5'→3'. Sequence data on listed species were obtained from the Antwerp ribosomal database. Nucleotides underlined and in boldface differ from corresponding nucleotides in amplimer.  
<sup>b</sup> Amplimer 27f sequence.  
<sup>c</sup> Amplimer 63f sequence.  
<sup>d</sup> Amplimer 1387r sequence.  
<sup>e</sup> Amplimer 1392r sequence.

and that systematic empirical testing of the amplimers is of paramount importance.

We thank Nyree West and Julie Scanlan for the kind gift of DNA from the five representatives of the cyanobacteria and chloroplast group and Guy Baranton for the three *Leptospira* strains.

We gratefully acknowledge support from the NERC through grant GR3/9490 (to J.R.M.) and a studentship (to T.A.M.).

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## ERRATA

### Methane and Trichloroethylene Oxidation by an Estuarine Methanotroph, *Methylobacter* sp. Strain BB5.1

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Volume 63, no. 11, p. 4618, Table 1: footnote *b* should read “Micromoles of CH<sub>4</sub> or nanomoles of TCE hour<sup>-1</sup> milligram of total protein<sup>-1</sup>.”

Page 4619, Fig. 3A and B, y axes: “nmol” should read “μmol.”

