JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1994, p. 335–351
0095-1137/94/$04.00+0

Vol. 32, No. 2

PCR Primers and Probes for the 16S rRNA Gene of Most Species of Pathogenic Bacteria, Including Bacteria Found in Cerebrospinal Fluid

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Received 21 July 1993/Returned for modification 29 September 1993/Accepted 1 November 1993

A set of broad-range PCR primers for the 16S rRNA gene in bacteria were tested, along with three series of oligonucleotide probes to detect the PCR product. The first series of probes is broad in range and consists of a universal bacterial probe, a gram-positive probe, a *Bacteroides-Flavobacterium* probe, and two probes for other gram-negative species. The second series was designed to detect PCR products from seven major bacterial species or groups frequently causing meningitis: *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. agalactiae*, *Escherichia coli* and other enteric bacteria, *Listeria monocytogenes*, and *Staphylococcus aureus*. The third series was designed for the detection of DNA from species or genera commonly considered potential contaminants of clinical samples, including cerebrospinal fluid (CSF): *Bacillus*, *Corynebacterium*, *Propionibacterium*, and coagulase-negative *Staphylococcus* spp. The primers amplified DNA from all 124 different species of bacteria tested. Southern hybridization testing of the broad-range probes with washes containing 3 M tetramethylammonium chloride indicated that this set of probes correctly identified all but two of the 102 bacterial species tested, the exceptions being *Deinococcus radiopugnans* and *Gardnerella vaginalis*. The gram-negative and gram-positive probes hybridized to isolates of two newly characterized bacteria, *Alloiococcus otitis* and *Rochalimaea henselii*, as predicted by Gram stain characteristics. The CSF pathogen and contaminant probe sequences were compared with available sequence information and with sequencing data for 32 different species. Testing of the CSF pathogen and contaminant probes against DNA from over 60 different strains indicated that, with the exception of the coagulase-negative *Staphylococcus* probes, these probes provided the correct identification of bacterial species known to be found in CSF.

Effective treatment of bacterial infections often requires the rapid and accurate detection and identification of bacteria in sterile body fluids, such as blood and cerebrospinal fluid (CSF). Among the procedures currently used in clinical laboratories, the most sensitive method of detecting bacteria is growth in a culture. Culturing requires at least an 8-h incubation of the sample in culture medium and then biochemical and/or immunological tests to identify the bacterium. The time required to obtain a positive culture result can be even longer for patients infected with slowly growing organisms or with low bacterial counts (because of the type of infection or prior antibiotic therapy). The most rapid tests available for CSF, latex agglutination tests, are less sensitive, with reliable results being obtained only for samples with over 10^5 CFU per ml (4, 21). Since as many as 45% of meningitis cases involve bacterial loads of less than 10^5 CFU per ml (4, 21), immunological tests are not sufficiently sensitive. In addition, the specificity of these tests is often inadequate. Because of the time required to detect bacteria and the mortality associated with untreated bacteremia and bacterial meningitis, antibiotic therapy is often prescribed empirically or prophylactically.

Assays based on nucleic acid detection have the potential for greater sensitivity than immunological assays. For example, Wilson et al. recently described an assay for bacteremia which was based on signal amplification by Q-beta replicase and which allowed the detection of 10 CFU of *Escherichia coli* per sample (40). The PCR has been applied, in numerous reports (38), to the detection and identification of specific bacterial pathogens. In many cases, the target genes are those known to be involved in the pathogenicity or virulence of the pathogen (15, 30). For example, Kuritza and Oehler described the use of a protease A gene for the detection of a specific bacterial species causing meningitis, *Neisseria meningitidis* (19). In other cases, the target is a random sequence cloned from a genomic library and selected by differential hybridization to the pathogen and its close relatives (35). Mahbubani et al. described a system for the detection of *Legionella pneumophila* and *Legionella* species with two sets of PCR primers in a multiplex reaction (25).

The use of universal PCR primers targeting DNA regions conserved in bacteria for the purpose of DNA sequencing or probe design has been described (5, 39). In this report, we describe the use of such primers for the detection and identification of the wide range of bacterial pathogens causing septicemia and meningitis. For example, a variety of bacterial species or groups other than *N. meningitidis* is frequently found in the CSF of patients with bacterial meningitis; these include *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. agalactiae*, *Listeria monocytogenes*, enteric bacteria, and *Mycobacterium tuberculosis*. PCR primers and probes were designed for the detection of bacteria in blood, CSF, and other normally sterile body fluids. The universal bacterial primers were tested against DNA from more than 100 bacterial strains, including the major bacterial species causing septicemia and meningitis and species or genera that are commonly considered con-

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taminants of clinical samples. In addition, we describe three series of probes that hybridize to the amplified PCR product and generate a specific signal. The first series (universal, gram-positive, and gram-negative oligonucleotide probes) was found to identify correctly the majority of the 102 bacterial species tested. The second series includes seven probes which detect DNA from the major bacterial causes of meningitis: *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, *S. agalactiae*, *E. coli* and other enteric bacteria, *L. monocytogenes*, and *Staphylococcus aureus*. The third series consists of five probes for species or genera which are commonly considered contaminants: *Bacillus*, *Corynebacterium*, *Propionibacterium*, and coagulase-negative *Staphylococcus* spp. In Southern hybridization testing, the second and third series of probes, with the exception of the coagulase-negative *Staphylococcus* probes, provided correct identification of over 60 different strains representing 18 different bacterial species found as pathogens or presumptive contaminants in human CSF.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study and their sources are listed in Tables 1 and 2. Additional strains were obtained from Howard Faden (State University of New York at Buffalo, Buffalo) and Dan Lucey (Walter Reed Army Institute for Research, Bethesda, Md.). Bacteria were obtained in the form of frozen cell pellets, streaks, or lyophilized cells. Prior to DNA extraction, each strain was streaked on chocolate or blood agar and examined for the proper colony morphology. In some cases, Gram staining was performed to confirm the identification.

DNA isolation. All DNA isolation procedures were done with positive-displacement pipettes to minimize strain-to-strain contamination. DNA was isolated from the strains in Table 1 by method 1, 2, or 3, each of which is described below; all of the DNA from strains in Table 2 was isolated by method 3. In method 1, DNA was isolated from *Corynebacterium pseudotuberculosis* by the lysozyme–sodium dodecyl sulfate (SDS)–proteinase K–phenol-chloroform method described by Patel et al. (31). Methods 2 and 3 are SDS–proteinase K–phenol-chloroform methods similar to the method of Silhavy et al. (37). In method 2, for staphylococci, lysostaphin at a final concentration of 5 U/ml was used; for other bacteria, lysozyme at a final concentration of 1 mg/ml was used. This method included a boiling step after the SDS-proteinase K treatment and an RNase step. In method 3, only lysozyme was used to aid in cell lysis, and no boiling or RNase step was used. Samples were resuspended in 50 μl of TE (10 mM Tris [pH 8.0], 0.1 mM EDTA) and run on 0.9% agarose gels to estimate the DNA concentrations.

DNA amplification. The locations of the universal primers RW01, RDR080, and DG74 within the 16S rRNA gene are indicated in Table 3. All oligonucleotides used in this study were provided by the DNA Synthesis Group, Roche Molecular Systems. DNA was amplified in a 100-μl reaction mixture consisting of 10 μl of 10× PCR buffer, 1 μl of 50 mM $MgCl_2$, 2.5 μl of 2.5 mM total deoxynucleoside triphosphates, 1 μl of 50 μM primer RW01 or RDR080, 1 μl of 50 μM primer DG74, 34 μl of H_2O, and 0.5 μl of Amplitaq DNA polymerase (10× PCR buffer contains 100 mM Tris-HCl [pH 8.3], 500 mM KCl, and 15 mM $MgCl_2$). For some reactions, 4 μl of 50% glycerol and 30 μl of H_2O were added in place of the 34 μl of H_2O. The mixture was placed in a thermal cycler, incubated at 95°C for 5 min, cycled 25 times for 25 s each time at 95 and 55°C, and incubated for 10 min at 72°C. After amplification, 5 μl of the amplified product was run on a 2% Nusieve–0.5% SeaKem agarose gel. The gel was blotted onto a Pall Biodyne membrane by the capillary method or by vacuum blotting with a Stratagene Vacublotter according to the manufacturer's directions. DNA was fixed to the membrane with a UV cross-linker (Stratagene).

Estimation of sensitivity. The sensitivity of amplification was tested with purified *E. coli* DNA and *E. coli* cells, both added directly to the amplification mixture. The DNA was quantitated spectrophotometrically and then serially diluted to the appropriate concentration. The cells were grown in Luria broth to log phase and serially diluted 10-fold in sterile water. Aliquots from the same dilutions were removed for amplification and for plating onto L agar. CFU were counted after overnight growth at 37°C. Reactions testing the sensitivity of amplification were done with 30 to 35 cycles rather than 25. Reagents (*Taq* buffer, nucleotides, and primers) were filtered through a Centricon-100 device prior to use. This treatment reduces the amount of endogenous DNA present in the reagents. In addition, the *Taq* polymerase preparation used in these experiments was treated to reduce the amount of contaminating DNA (this preparation was obtained from the Development Department, Roche Molecular Systems, and is now available from Perkin-Elmer as AmpliTaq DNA polymerase, LD).

DNA sequencing and sequence analysis. DNAs to be sequenced were amplified with primers PL06 and DG74. Ten-nanogram amounts of total bacterial DNA were amplified in two different ways. One set of PCRs used biotinylated PL06 and nonbiotinylated DG74, whereas the other set of PCRs used nonbiotinylated PL06 and biotinylated DG74. The conditions used for the amplifications were those described above, except that the number of cycles was 25 and an annealing temperature of 60°C rather than 55°C was used for DNAs from all species except *H. influenzae*.

For the preparation of single-stranded DNA to use as a template in the sequencing reactions, two different methods, both based on that of Mitchell and Merrill (27), were used. One method was based on the use of streptavidin linked to magnetic beads (6). The second method used streptavidin-agarose beads (Bethesda Research Laboratories, catalog no. 5942SA) instead of magnetic beads. The procedure used was the same as that for the first method, with the following exceptions. After the ammonium acetate addition, the streptavidin-agarose beads were spun in a microcentrifuge for 5 min at 13,000 × *g* to pellet any debris. The supernatant (480 μl) was transferred to a new microcentrifuge tube, leaving 20 μl left in the original tube. This pelleting step was repeated once more, with 460 μl being transferred to the new tube and 20 μl being left in the original tube. After the three Centricon-100 washes of the neutralized single strands (6), the Centricon-100 top reservoir was inverted, 20 μl of water was added, and the tube was spun for a short time at 1,000 rpm in a Sorvall SS34 centrifuge rotor. For both methods, the single-stranded DNA obtained was resuspended in 10 μl of H_2O. Four to 7.5 μl of the solution was used in a Sequenase sequencing reaction with dGTP and/or inosine.

DNA sequences were either read manually and entered into the GENED program (Intelligenetics) or recorded into Gelread (Intelligenetics) by use of a semiautomated DNA sequencing gel reader (Bio-Rad, Richmond, Calif.). Sequences were analyzed with the University of Wisconsin GCG program Find, Intelligenetics programs Fold, Genalign, and Findseq, the IRX program from the GenBank Online Service, the OLIGO 3.4 program (National Bio-

TABLE 1. Strains, isolation methods, and amplification hybridization results with broad-range probes

Organism	Strain or source[a]	Isolation method[b]	Amplification[c]	Result[d] with the following probe:					
				Gram positive (RW03)	Gram negative (DL04)	Gram negative (RDR278)	Bacteroides (RDR279)	Universal (RDR245)	Enteric (RDR140)
Gram negative									
Acinetobacter calcoaceticus	ATCC 23055	2	+	−	+	±	−	+	−
A. lwoffii	ATCC 15309	2	+	−	+	±	−	+	−
Achromobacter xerosis	ATCC 14780	2	+	−	+	±	−	+	−
Aeromonas hydrophila	ATCC 7966	2	+	−	+	±	−	+	−
Agrobacterium radiobacter	ATCC 19358	3	+	−	±	+	−	+	−
Alcaligenes denitrificans	ATCC 27061	2	+	−	−	+	−	+	−
A. faecalis	ATCC 8750	2	+	−	−	+	−	+	−
Bacteroides fragilis	ATCC 25285	2	+	−	−	−	+	+	−
Campylobacter fetus	ATCC 27374	3	*	−	+	+	−	+	ND
C. jejuni	ATCC 33560	3	*	−	+	+	−	+	ND
Chromobacterium violaceum	ATCC 12472	2	+	−	−	+	−	+	−
Citrobacter freundii	ATCC 8090	2	+	−	+	±	−	+	+
Derxia gummosa	ATCC 15994	2	+	−	−	+	−	+	−
Edwardsiella tarda	ATCC 15947	2	+	−	+	±	−	+	+
Eikenella corrodens	ATCC 23834	2	+	−	−	+	−	+	−
Enterobacter aerogenes	ATCC 13048	3	+	−	+	ND	ND	ND	ND
E. cloacae	ATCC 13047	2	+	−	+	±	−	+	+
Escherichia coli	ATCC 11775	2	+	−	+	±	−	+	+
Flavobacterium meningosepticum	ATCC 13253	3	±	−	−	−	+	+	−
Haemophilus ducreyi	ATCC 33940	2	+	−	+	±	−	+	−
H. influenzae	ATCC 33391	2	+	−	+	±	−	+	−
H. influenzae	503-1156	3	+	−	+	±	−	+	ND
Kingella kingae	ATCC 23330	2	+	−	+	±	−	+	ND
Klebsiella pneumoniae	ATCC 13883	3	+	−	+	±	−	+	ND
K. pneumoniae	CMCC 151	3	+	−	+	ND	ND	ND	ND
Legionella bozemanii	ATCC 33217	2	+	−	+	±	−	+	−
L. pneumophila	ATCC 33152	2	+	−	+	±	−	+	−
Moraxella catarrhalis	ATCC 25238	3	+	−	+	±	−	+	−
M. osloensis	ATCC 19976	2	+	−	+	±	−	+	−
Morganella morganii	ATCC 25830	3	+	−	+	±	−	+	ND
Neisseria gonorrhoeae	ATCC 19424	2	+	−	−	+	−	+	−
N. meningitidis	ATCC 13077	2	+	−	−	+	−	+	−
Paracoccus denitrificans	ATCC 17741	2	+	−	+	+	−	+	−
Proteus mirabilis	ATCC 29906	2	+	−	+	±	−	+	+
Providencia stuartii	ATCC 29914	2	+	−	+	±	−	+	+
Pseudomonas aeruginosa	ATCC 10145	2	+	−	+	±	−	+	−
P. aeruginosa	ATCC 27853	3	+	−	+	ND	ND	ND	ND
P. putida	ATCC 12633	2	+	−	+	±	−	+	−
Rahnella aquatilis	ATCC 33071	2	+	−	+	±	−	+	+
Rhodospirillum rubrum	ATCC 11170	2	+	−	−	±	−	+	−
Salmonella typhimurium	CMCC 2	3	+	−	+	ND	ND	ND	ND
Serratia marcescens	ATCC 13880	2	+	−	+	±	−	+	+
S. marcescens	CMCC 186	3	+	−	+	ND	ND	ND	ND
Shigella boydii	CMCC	3	+	−	+	ND	ND	ND	ND
S. dysenteriae	CMCC	3	+	−	+	ND	ND	ND	ND
S. flexneri	CMCC	3	+	−	+	ND	ND	ND	ND
S. sonnei	CMCC	3	+	−	+	ND	ND	ND	ND
Vibrio parahaemolyticus	ATCC 17802	2	±	−	±	−	−	+	ND
Yersinia enterocolitica	ATCC 9610	2	+	−	+	±	−	+	+
Gram positive and others									
Actinomyces israelii	ATCC 12102	3	*	+	−	−	−	+	ND
Aerococcus viridans	ATCC 11563	2	+	+	−	−	−	+	−
Bacillus amyloliquefaciens	CMCC-H	3	+	+	−	−	−	ND	ND
B. subtilis	ATCC 6051	2	+	+	−	−	−	+	−
B. subtilis	CMCC	3	+	+	−	−	−	ND	ND
Bifidobacterium adolescentis	ATCC 15703	2	+	+	−	−	−	+	−
Brevibacterium linens	ATCC 9172	2	+	+	−	−	−	+	−
Clostridium innocuum	ATCC 14501	2	+	+	−	−	−	+	−
C. perfringens	ATCC 13124	2	+	+	−	−	−	+	−
C. perfringens	Sigma	NA	+	+	−	ND	ND	ND	ND
Corynebacterium genitalium	ATCC 33030	3	+	+	−	−	−	+	−
C. pseudotuberculosis	ATCC 19410	1	+	+	−	−	−	+	−
C. xerosis	ATCC 373	3	+	+	−	−	−	+	ND

Continued on following page

TABLE 1—*Continued*

Organism	Strain or source[a]	Isolation method[b]	Amplification[c]	Result[d] with the following probe: Gram positive (RW03)	Gram negative (DL04)	Gram negative (RDR278)	Bacteroides (RDR279)	Universal (RDR245)	Enteric (RDR140)
C. jeikeium	ATCC 43734	3	+	+	−	−	−	+	ND
Deinococcus radiopugnans	ATCC 19172	2	+	−	−	−	−	+	−
Enterococcus avium	ATCC 14025	2	+	+	−	−	−	+	−
E. faecalis	ATCC 19433	2	+	+	−	−	−	+	−
E. faecium	ATCC 19434	2	+	+	−	−	−	+	−
Erysipelothrix rhusiopathiae	ATCC 19414	2	+	+	−	−	−	+	−
Gardnerella vaginalis	ATCC 14018	2	+	−	−	−	−	+	−
Gemella haemolysans	ATCC 10379	3	+	+	−	−	−	+	ND
Lactobacillus acidophilus	ATCC 4356	2	+	±	−	−	−	+	−
L. brevis	ATCC 14869	2	+	+	−	−	−	+	−
L. jensenii	ATCC 25258	2	+	+	−	−	−	+	−
Lactococcus lactis subsp. *cremoris*	ATCC 19257	2	+	+	−	−	−	+	−
L. lactis subsp. *lactis*	ATCC 19435	2	+	+	−	−	−	+	−
Leuconostoc paramesenteroides	ATCC 33313	2	+	+	−	−	−	+	−
Listeria monocytogenes	ATCC 15313	2	+	+	−	−	−	+	−
Micrococcus luteus	ATCC 4698	2	*	+	−	−	−	+	ND
M. lysodeikticus	Sigma	NA	±	±	−	ND	ND	ND	ND
Mycobacterium bovis	CMCC	3	±	+	−	ND	ND	ND	ND
M. gordonae	ATCC 14470	3	+	+	−	−	−	+	ND
M. smegmatis	ATCC 19420	3	+	+	−	−	−	+	−
M. tuberculosis	CMCC	3	±	+	−	ND	ND	ND	ND
Mycoplasma genitalium	ATCC 33530	3	+	+	−	−	−	+	−
M. hominis	ATCC 23114	2	+	+	−	−	−	+	−
M. pneumoniae	ATCC 15531	2	±	+	−	−	−	+	−
Pediococcus acidilactici	ATCC 33314	2	+	+	−	−	−	+	−
Peptostreptococcus anaerobius	ATCC 27337	2	+	+	−	−	−	+	−
P. magnus	ATCC 15794	3	+	+	−	−	−	+	−
Propionibacterium acnes	ATCC 6919	2	+	+	−	−	−	+	−
P. lymphophilum	ATCC 27520	3	*	+	−	−	−	+	ND
Staphylococcus aureus	ATCC 12598	2	+	+	−	−	−	+	−
S. aureus	ATCC 33589	2	+	+	−	ND	ND	ND	−
S. aureus	ATCC 25923	3	+	+	−	ND	ND	ND	ND
S. epidermidis	ATCC 14990	2	+	+	−	−	−	+	−
Streptococcus agalactiae	ATCC 13813	2	+	+	−	−	−	+	−
S. bovis	ATCC 33317	2	+	+	−	−	−	+	−
S. dysgalactiae	ATCC 43078	2	+	+	−	−	−	+	−
S. equinus	ATCC 9812	2	+	+	−	−	−	+	−
S. intermedius	ATCC 27335	2	+	+	−	−	−	+	−
S. mitis	ATCC 33399	2	+	+	−	−	−	+	−
S. mutans	ATCC 25175	2	+	+	−	−	−	+	−
S. pneumoniae	ATCC 33400	2	+	+	−	−	−	+	−
S. pyogenes	ATCC 12344	2	+	+	−	−	−	+	−
S. salivarius	ATCC 13419	2	+	+	−	−	−	+	−
S. sanguis	ATCC 10556	2	+	+	−	−	−	+	−
S. uberis	ATCC 19436	2	+	+	−	−	−	+	−
Streptomyces griseinus	ATCC 23915	3	*	+	−	−	−	+	ND
S. hygroscopicus	ATCC 21705	3	*	+	−	−	−	+	ND
Ureaplasma urealyticum	ATCC 27618	3	+	+	−	−	−	+	−

[a] All strains isolated by methods 1 and 2 were obtained from M. Loeffelholz and A. Purohit. Other ATCC strains were obtained from the American Type Culture Collection. Strains designated CMCC were obtained from the Cetus culture collection. Strain 503-1156 was obtained from G. Beskid, Hoffmann-La Roche Inc., Nutley, N.J.

[b] See Materials and Methods. NA, not applicable.

[c] *, the strain was amplified better with RDR080 than with RW01.

[d] ND, not done.

sciences, Plymouth, Minn.), and the ESEE program (designed by E. L. Cabot and A. T. Beckenbach).

Probe hybridization. Oligonucleotides were labeled with ^{32}P by use of T4 polynucleotide kinase (New England Biolabs). Probes (1×10^6 to 2×10^6 cpm/10 ml) were hybridized to DNA blots in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–0.5% SDS at 60°C for 1.5 to 18 h. Blots were washed once at room temperature in 2× SSPE–0.1% SDS and once at room temperature in tetramethyl-ammonium chloride (TMACl) solution (3 M TMACl, 50 mM Tris-HCl [pH 8.0], 0.2% SDS) (42). Stringent washes in TMACl were done at the temperatures indicated in Table 3.

DNA sequence accession numbers. The Genbank accession numbers for the determined sequences (see Fig. 3) are as follows: *Propionibacterium acnes*, U02904; *P. avidum*, U02905; *P. lymphophilum*, U02907; *P. granulosum*, U02906; *C. xerosis*, U02895; *S. epidermidis*, U02913; *S. auricularis*,

TABLE 2. CSF pathogens and contaminants and amplification results

Organism	Strain	Source[a]	Amplification
Haemophilus influenzae	ATCC 33391	C	+
	2423	N	+
	503-1156	N	+
	503-1148	N	+
	503-1155	N	+
	503-1154	N	+
Streptococcus pneumoniae	ATCC 33400	ML	+
	ATCC 6303	B	+
	4366	B	+
	4471	B	+
S. agalactiae	ATCC 13813	B	+
	4352	B	±
	4353	B	+
	4354	B	+
	4355	B	+
	4356	B	+
S. salivarius	ATCC 13419	ML	+
	ATCC 7073	B	+
S. equi	NCTC 9682	B	+
Streptococcus group G	4286	B	+
S. pyogenes	ATCC 19615	B	+
S. dysgalactiae	ATCC 43078	ML	+
S. anginosus	ATCC 12395	B	+
S. milleri	4221	B	+
S. mitis	NCTC 3165	B	+
S. mutans	ATCC 25175	B	+
S. sanguis	ATCC 10556	B	+
S. intermedius	ATCC 27335	ML	+
Neisseria meningitidis	CMCC 2801	C	+
N. meningitidis	ATCC 13077	ML	+
N. meningitidis serotype A		CDC	+
N. meningitidis serotype B		CDC	+
N. meningitidis serotype C		CDC	+
N. meningitidis serotype Y		CDC	+
N. meningitidis serotype W135		CDC	+
N. gonorrhoeae	CMCC 2783	C	+
	ATCC 19424	ML	+
	31917	N	+
	31959	N	±
	32171	N	+
	32213	N	+
N. gonorrhoeae subsp. *kochii*	NRL 32895	CDC	+
	NRL 32896	CDC	+
	NRL 31291	CDC	+
	NRL 31292	CDC	+
	NRL 31294	CDC	+
	CDC 10,046	CDC	+
N. cinerea	CDC 10,050	CDC	+
	CDC 10,051	CDC	+
	CDC 10,052	CDC	+
	CDC 10,053	CDC	+
	CDC 10,054	CDC	+
N. sicca	Clinical isolate	Rush	+
N. polysaccharea	ATCC 43768	ATCC	+
	CDC 10,049	CDC	+
	CDC 10,048	CDC	+
	CDC 10,047	CDC	+
Eikenella corrodens	ATCC 23834	ATCC	+
Escherichia coli	Strain B	Sigma	+
	ATCC 11775	ML	+
	9	N	+
	P3478	N	+
	2889	N	+
	340	N	+
Listeria monocytogenes	ATCC 15313	ML	+
L. monocytogenes serotype 1/2a	G0282	CDC	+
L. monocytogenes serotype 1/2c	G0288	CDC	+
L. monocytogenes serotype 1/2b	F9784	CDC	+
L. monocytogenes serotype 4b	G0278	CDC	+

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TABLE 2—*Continued*

Organism	Strain	Source[a]	Amplification
L. monocytogenes serotype 3b	F9841	CDC	+
Bacillus subtilis	BD224	C	+
	ATCC 6051	N	+
	558	N	+
B. cereus	ATCC 11778	N	+
B. amyloliquefaciens	H	C	+
B. pumilis	ATCC 72	ATCC	+
B. brevis	ATCC 8186	ATCC	+
	ATCC 8246	ATCC	+
Corynebacterium genitalium	ATCC 33030	ML	+
C. jeikeium	ATCC 43734	ATCC	+
C. pseudotuberculosis	ATCC 19410	ML	+
C. xerosis	ATCC 373	ML	+
Propionibacterium acnes	ATCC 6919	ML	+
P. avidum	ATCC 25577	ATCC	+
P. granulosum	ATCC 25564	ATCC	+
P. lymphophilum	ATCC 27520	ATCC	+
Staphylococcus epidermidis	ATCC 12228	B	+
	ATCC 14990	ML	+
	4233	B	+
	4234	B	+
	4235	B	+
	4236	B	+
S. aureus	ATCC 33589	ML	+
	ATCC 25923	B	+
	4241	B	+
	4247	B	+
	4248	B	+
	4249	B	+
S. auricularis	ATCC 33753	ATCC	+
S. capitis subsp. *capitis*	ATCC 35661	ATCC	+
S. cohnii subsp. *cohnii*	ATCC 35662	ATCC	+
S. haemolyticus	ATCC 29970	ATCC	+
S. hominis	ATCC 29885	ATCC	+
S. saprophyticus	ATCC 15305	ATCC	+
S. warneri	ATCC 27836	ATCC	+
S. saccharolyticus	ATCC 14953	ATCC	+
Flavobacterium meningosepticum	ATCC 13253	ML	±

[a] Strains designated CDC were obtained from B. Swaminathan and Joan Knapp, Centers for Disease Control and Prevention, Atlanta, Ga.; strains designated B were obtained from Isolde Birk and Sylvain Rueff, Hoffmann-La Roche, Basel, Switzerland; strains designated N were obtained from G. Beskid, Hoffmann-La Roche Inc., Nutley, N.J.; strains designated C were obtained from the Cetus culture collection; strains designated ML were obtained from M. Loeffelholz and A. Purohit; strains designated ATCC were obtained from the American Type Culture Collection; and strains designated Rush were obtained from A. Kuritza, Rush-St. Luke's-Presbyterian Medical Center, Chicago, Ill.

U02911; *S. saccharolyticus*, U02922; *S. aureus*, U02910; *Bacillus pumilis*, U02894; *B. cereus*, U02893; *Flavobacterium meningosepticum*, U02896; *S. mitis*, U02918; *S. mutans*, U02919; *S. equi*, U02914; *Streptococcus* group G, U02915; *S. pyogenes*, U02921; *S. dysgalactiae*, U02912; *S. salivarius*, U02923; *S. anginosus*, U02909; *S. milleri*, U02917; *S. sanguis*, U02924; *S. intermedius*, U02916; *S. agalactiae*, U02908; *S. pneumoniae*, U02920; *N. meningitidis* serotype A, U02901; *N. polysaccharea*, U02902; *N. gonorrhoeae* subsp. *kochii*, U02900; *N. cinerea*, U02899; *N. sicca*, U02903; *H. influenzae*, U02897; and *L. monocytogenes*, U02898.

RESULTS

Specificity of universal bacterial primers. Primers RW01, RDR080, and DG74 correspond to regions of the 16S rRNA gene which are highly conserved among divergent groups of eubacteria and therefore would be expected to amplify DNA from most pathogenic bacteria. The primer locations were chosen to be relatively specific for eubacterial genes; at the 3′ ends, there are numerous mismatches with small-subunit rRNA genes (nuclear and mitochondrial) found in eukaryotes such as humans and fungi. The RW01 and DG74 primers were initially tested against DNA from a panel of 102 bacterial species (Table 1). The panel included gram-negative and gram-positive bacteria as well as spirochetes and mycoplasmas. Amplification was observed for all of the species tested, with DNAs from 12 species being amplified less well than DNAs from the rest (*Actinomyces israelii*, *Campylobacter jejuni*, *C. fetus*, *F. meningosepticum*, *Vibrio parahaemolyticus*, *Micrococcus luteus*, *M. lysodeikticus*, *M. bovis*, *M. tuberculosis*, *Mycoplasma pneumoniae*, *Streptomyces griseinus*, and *S. hygroscopicus*).

DNAs from some of the above-mentioned species were not amplified well with RW01, even though they were predicted to have a perfect match for the 3′ end of RW01: *A. israelii* (X53228), *F. meningosepticum* (M58776), *M. bovis* (M20940 and X55589), *M. tuberculosis* (X52917 and X55588), and *M. pneumoniae* (M29061). The 16S genes of some of the other species were expected to have a 3′-end mismatch for primer RW01 on the basis of available sequence data; these included *Campylobacter* species (*C.*

TABLE 3. Nucleotide sequences and locations of primers and probes

Primer or probe	Sequence	Location[a]	Wash (°C)[b]
Universal bacterial primer			
DG74	5'-AGGAGGTGATCCAACCGCA-3'	1522–1540	NA
RW01	5'-AACTGGAGGAAGGTGGGGAT-3'	1170–1189	NA
RDR080	5'-AACTGGAGGAAGGTGGGGAC-3'	1170–1189	NA
PL06	5'-GGTTAAGTCCCGCAACGAGCGC-3'	1088–1109	NA
Universal bacterial probe, RDR245	5'-GTACAAGGCCCGGGAACGTATTCACCG-3'	1369–1395	64
Gram-positive universal probe, RW03	5'-GACGTCAAATCATCATGCCCCTTATGTC-3'	1190–1217	64
Gram-negative probe			
DL04	5'-GACGTAAGGGCCATGATGACTTGACGTC-3'	1190–1217	64
RDR278	5'-GACGTAAGGGCCATGAGGACTTGACGTC-3'	1190–1217	64
Bacteroides-Flavobacterium probe, RDR279	5'-GACGTAAGGGCCGTGCTGATTTGACGTC-3'	1190–1217	64
Haemophilus species probe, RDR125	5'-GGAGTGGGTTGTACCAGAAGTAGAT-3'	1416–1440	66
Streptococcus pneumoniae probe, RDR462	5'-AACTGAGACTGGCTTTAAGAGATTA-3'	1278–1302	64–66
Escherichia coli-enteric bacterium probe, RDR140	5'-GGCGCTTACCACTTTGTGATTCATG-3'	1458–1482	66
Listeria monocytogenes probe, RDR230	5'-CTAATCCCATAAAACTATTCTCAGT-3'	1277–1301	64
Streptococcus agalactiae probe, KG0001	5'-TAATCTCTTAAAGCCAATCTCAGTT-3'	1278–1302	64–66
Neisseria meningitidis probe, COR28	5'-AAGCCGCGAGGCGGAGCCAATCT-3'	1261–1283	64
Bacillus probe, RDR502	5'-GTATTCACCGCGGCATGCTGATCCG-3'	1354–1378	66
Corynebacterium probe, RDR510	5'-ACTGTACCGACCATTGTAGCATGTG-3'	1228–1252	66
Propionibacterium probe, RDR514	5'-GGTGTGTACAAGCCCCGGGAACGTA-3'	1376–1400	66
Coagulase-negative *Staphylococcus* probe I, RDR325	5'-CGACGGCTAGCTCCAAATGGTTACT-3'	1443–1467	66
Coagulase-negative *Staphylococcus* probe II, RDR512	5'-CGGCTAGCTCCAAAAGGTTACTCTA-3'	1440–1464	64–66
Staphylococcus aureus probe, RDR327	5'-GCCGGTGGAGTAACCTTTTAGGAGC-3'	1435–1458	66

[a] Nucleotide numbering is as for *E. coli*.
[b] NA, not applicable.

jejuni [M59298], *C. fetus* subsp. *veneralis* [M65011], and *C. fetus* subsp. *fetus* [M65012]) and *V. parahaemolyticus* (M59161). In addition, *M. luteus* (M38242) was predicted to have an additional mismatch located 5 bases from the 3' end of the primer. RDR080 was designed so that its 3' end would be a perfect match for the *C. jejuni*, *C. fetus* subsp. *veneralis*, *C. fetus* subsp. *fetus*, and *V. parahaemolyticus* genes.

DNAs from some of the above-mentioned species (*A. israelii*, *C. jejuni*, *C. fetus*, *F. meningosepticum*, *V. parahaemolyticus*, *M. luteus*, *S. griseinus*, and *S. hygroscopicus*) were tested with primer RDR080 instead of RW01 in amplifications with DG74. As predicted, *C. jejuni* and *C. fetus* DNAs gave higher PCR product yields with RDR080 than with RW01. *A. israelii*, *M. luteus*, *S. griseinus*, and *S. hygroscopicus* also gave higher PCR product yields with RDR080 than with RW01. The remaining DNAs (*F. meningosepticum* and *V. parahaemolyticus*) gave the same or lower levels of amplification with RDR080.

Universal bacterial probe. The universal bacterial probe RDR245 was designed from a conserved region of the 16S rRNA gene which is located between the universal bacterial primers, at bp 1369 to 1395 (antisense) of the *E. coli* gene (Table 3). The PCR products from amplifications with RW01 and DG74 were run on agarose gels and blotted onto nylon membranes, and the blots were hybridized with radioactively labeled oligonucleotide RDR245, with washes done in the presence of 3 M TMACl. The results are shown in Table 1. All of the PCR products hybridized with the probe, the intensity of the signal varying roughly with the amount of DNA present on the blot.

Universal gram-positive and gram-negative probes. The gram-positive universal probe RW03 corresponds to two sequence signatures identified in 16S rRNA by Woese and coworkers (41). The first sequence signature, present in all the gram-positive bacteria tested and absent in the gram-negative bacteria, is a C residue located at bp 1207 of the *E. coli* 16S rRNA gene. In addition, the probe corresponds to an A residue located at bp 1198 and present in 75% of the gram-positive species examined. Since there is no single signature which is present in all the bacteria classified as gram negative, the gram-negative universal probes DL04, RDR278, and RDR279 were designed to correspond to sequences unique to various subgroups of gram-negative bacteria.

The gram-negative and gram-positive probes were tested as the universal bacterial probe was (Table 1). The gram-positive probe appeared to be quite specific, detecting 55 of 57 gram-positive or other (gram-variable or nonstaining) species. The probe failed to detect *Deinococcus radiopugnans* and *Gardnerella vaginalis*.

The gram-negative probe DL04 hybridized to DNAs from 35 of 45 gram-negative species tested. As expected, DNAs from some of the gram-negative species did not hybridize to DL04. DNAs from 36 of 37 gram-negative species tested hybridized to RDR278, including DNAs from 8 of the 10 species which failed to hybridize to DL04. The only gram-negative species whose DNA did not hybridize to either DL04 or RDR278 were *Bacteroides fragilis* and *F. meningosepticum*. Neither DL04 nor RDR278 hybridized to any of the DNAs from the gram-positive species tested, including *D. radiopugnans* and *G. vaginalis*.

The RDR279 probe, designed to detect *Bacteroides* and *Flavobacterium* species, was highly specific, hybridizing only to *B. fragilis* and *F. meningosepticum* DNAs among the gram-negative and gram-positive species tested. The combination of DL04, RDR278, and RDR279 detected all 45 of the gram-negative and none of the 57 gram-positive or other species tested.

Sensitivity of universal primers. The sensitivity of detection of amplification with the universal primers was examined. Serial 10-fold dilutions of *E. coli* cells and DNA were

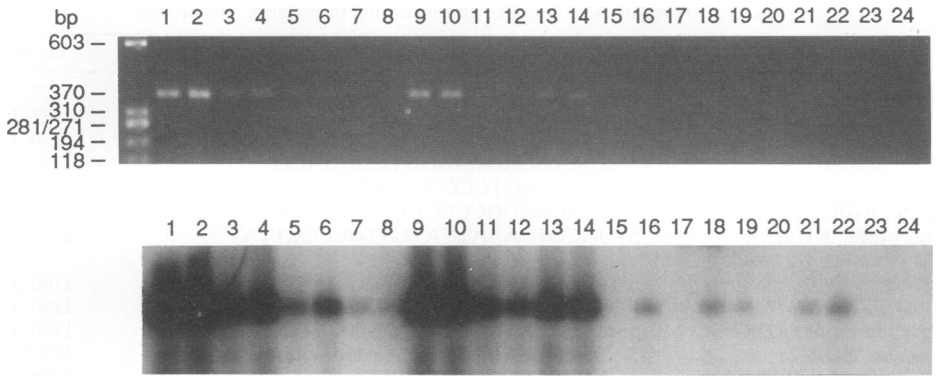

FIG. 1. Sensitivity of detection of *E. coli* cells. Serial dilutions of *E. coli* DNA and cells were amplified with primers RW01 and DG74 and run on an agarose gel (top panel). Reaction mixtures contained an estimated 1,000 copies of the 16S rRNA gene (lanes 1 and 2), 100 copies (lanes 3 and 4), 10 copies (lanes 5 and 6), and 1 copy (lanes 7 and 8); an estimated 320 CFU (lanes 9 and 10), 32 CFU (lanes 11 to 14), 3.2 CFU (lanes 15 to 18), and 0.3 CFU (lanes 19 to 22); and no DNA (lanes 23 and 24). A Southern blot of the gel was hybridized with ^{32}P-labeled *E. coli*-enteric bacterium probe RDR140 (bottom panel). Molecular marker sizes are given on the left in base pairs.

made and added to the reaction mixture (Fig. 1). At 35 cycles, it was possible to detect reproducibly a band in ethidium bromide-stained gels at dilutions corresponding to 30 CFU, or 100 copies of the *E. coli* 16S rRNA gene. Dilutions corresponding to lower cell numbers yielded a band sporadically, probably because of stochastic variations in the actual numbers of cells present in the volume of the dilution sampled. By hybridization with the *E. coli*-enteric bacterium probe RDR140, described below, it was possible to detect 3 CFU, or 10 copies.

Testing of DNA from novel bacteria. The DG74 and RW01 primers and the broad-range probes were used to obtain an initial characterization of two novel bacterial strains obtained from two sources. A novel gram-positive organism from middle ear effusions was characterized by Faden and Dryja (14) and was subsequently named *Alloiococcus otitis* (1). The other strain was an unknown gram-negative species isolated from blood and bone marrow cultures of samples from a patient with febrile illness and an episode of aseptic meningitis (23). This strain was identified as a *Rochalimaea henselii* strain on the basis of biochemical tests and DNA sequence analysis (24).

DNAs were isolated from the cells along with extraction controls as described previously (24). As shown in Fig. 2, all of the DNAs were amplified well. The DNAs were blotted, and probes RW03, DL04, and RDR278 were hybridized to separate blots. The blots included gram-negative controls *E. coli* and *Klebsiella pneumoniae* and gram-positive controls *Aerococcus viridans* and *Gemella haemolysans*. The results indicated that the *A. otitis* strain DNA hybridized as expected for an organism which stains gram positive. The *R. henselii* strain DNA also hybridized as expected, confirming its gram-negative nature.

CSF pathogen and contaminant probe design. Each of the CSF pathogen and contaminant probes was designed on the basis of nucleotide sequence information for the species or genera to be detected as well as for closely related species. The sequence information was obtained from either the GenBank or the EMBL data base or experimentally as described below. Sequence data were obtained experimentally for one isolate each of 32 species for an approximately 450-bp region between sequencing primers PL06 and DG74, corresponding to bp 1088 to 1540 in the *E. coli* 16S rRNA gene. The sources of the isolates are as listed in Table 2. For species with multiple isolates listed, the following isolates were used: *S. epidermidis* ATCC 14990, *S. aureus* ATCC 25923, *S. agalactiae* ATCC 13813, *S. salivarius* ATCC 7073, *S. pneumoniae* ATCC 6303, *N. meningitidis* serotype A CMCC 2801, *N. polysaccharea* ATCC 43768, *N. gonorrhoeae* subsp. *kochii* NRL 32895, *N. cinera* CDC 10050, *H. influenzae* ATCC 33391, and *L. monocytogenes* ATCC 5867. An alignment of the new sequences obtained is shown in Fig. 3. In many cases, a previously published sequence either had N's in areas which were readable on our sequencing gels or was missing information at the 5′ end of the amplified region or both (GenBank entries for *L. monocytogenes* [M58822], *B. cereus* [X55060 and X55063], *B. pumilis* [X60637], *S. mutans* [X58303], *S. equi* [X58314], *S. anginosus* [X58309], *S. salivarius* [X58320], *S. agalactiae* [X59032], *S. pyogenes* [X59029], *S. intermedius* [X58311], and *H. influenzae* [M35019]).

Two general regions of variability among all of the species analyzed were noted and correspond to variable regions 8 and 9. Candidate probes were chosen on the basis of the locations of mismatches with species that were to be excluded from detection by use of DNA sequence alignments and by running the GCG Find program against a GCG Strings file of 16S and small-subunit rRNA sequences. The candidate probes were then tested for secondary structure with the Fold program or the OLIGO 3.4 program.

Probes were designed for the following species, known to be major causes of meningitis: *S. pneumoniae*, *H. influenzae*, *S. agalactiae*, *L. monocytogenes*, *E. coli*-enteric bacteria, *N. meningitidis*, and *S. aureus*. In addition, probes were designed for the following common contaminant organisms: *Corynebacterium*, *Bacillus*, *Propionibacterium*, and coagulase-negative *Staphylococcus* spp. Each probe was designed to provide the correct identification of bacterial species or genus mainly within the group of bacteria commonly found in CSF. For example, the sequence differences between *N. meningitidis* and *N. gonorrhoeae* in the region flanked by the universal primers were not sufficient to allow the design of an *N. meningitidis* probe which did not also hybridize with *N. gonorrhoeae*. *N. gonorrhoeae*, however, is only rarely found in CSF, and the antibiotic used to treat the infection would be the same for both species. Given these two qualifications, the probes were designed to be capable of identifying the appropriate species in the majority of CSF samples tested.

Amplification of DNA from bacteria found in CSF. The

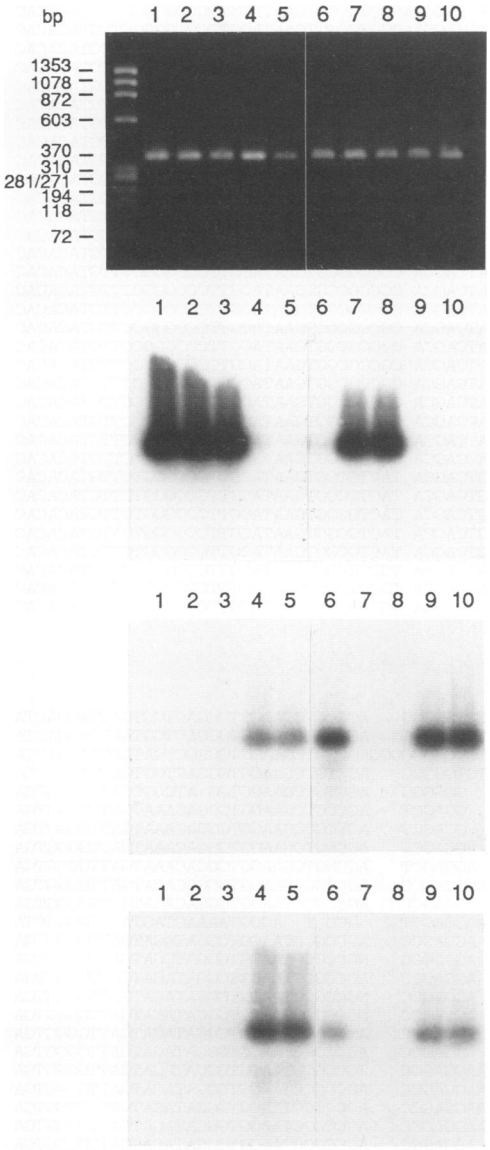

FIG. 2. Amplification and hybridization results with novel strains. Various bacterial strains were amplified with RW01 and DG74, and the products were run on an agarose gel: *A. otitis* 4419 (lane 1), *A. otitis* 7213 (lane 2), *A. otitis* 7760 (lane 3), *R. henselii* (lanes 4 and 5), *E. coli* (lane 6), *A. viridans* (lane 7), *G. haemolysans* (lane 8), *K. pneumoniae* ATCC 9997 (lane 9), and *K. pneumoniae* ATCC 27736 (lane 10) (first panel). Replicate Southern blots were probed with ^{32}P-labeled gram-positive probe RW03 (second panel), gram-negative probe DL04 (third panel), and gram-negative probe RDR278 (fourth panel). Molecular marker sizes are given in base pairs.

universal primers DG74, RW01 and, in some cases, RDR080 were used to amplify DNAs from the following 18 species of bacteria either found in CSF or closely related to species which are found in CSF (Table 4): *H. influenzae*, *S. pneumoniae*, *S. salivarius*, *S. agalactiae*, *N. meningitidis*, *N. gonorrhoeae*, *E. coli*, *L. monocytogenes*, *B. subtilis*, *B. cereus*, *B. amyloliquefaciens*, *C. genitalium*, *C. pseudotuberculosis*, *C. xerosis*, *P. acnes*, *S. epidermidis*, *S. aureus*, *F. meningosepticum*; additional species shown in Tables 2 and 5 were also used. With DG74 and either RDR080 or RW01, all of the amplification reactions produced a visible band on agarose gels stained with ethidium bromide. *P. lymphophilum* produced a higher yield of PCR products with RDR080 than with RW01. The nucleotide sequence data obtained for *P. lymphophilum* (Fig. 3) indicate that there are two mismatches at the 3′ end of the primer, one located 5 bases from the end of the primer and the other located at the very end.

Hybridization testing of CSF probes. The 12 CSF probes were each tested against the panel of 60 amplified DNAs (Table 4) as described above. The washes in the presence of 3 M TMACl were done at the temperatures given in Table 3. The results are summarized in Table 4. Within this panel, each of the probes hybridized specifically to the DNA of the species or genus for which it was designed and not to that of any other species or genus.

The specificity observed was in some cases sufficient to allow the differentiation of a single base-pair mismatch between the probe and the PCR product. For example, the *S. pneumoniae* and *S. agalactiae* probe sequences differ by a single base. The hybridization results indicated that the *S. pneumoniae* probe detected the PCR product from *S. pneumoniae* but not *S. agalactiae*. Similarly, the *S. agalactiae* probe detected the PCR product from *S. agalactiae* but not *S. pneumoniae*. Single base-pair discrimination was also observed for the *N. meningitidis* probe against *N. polysaccharea*, *N. cinerea*, and *N. sicca* DNAs, as described below.

The *N. meningitidis*, *S. pneumoniae*, *S. agalactiae*, *E. coli*-enteric bacterium, *S. aureus*, and coagulase-negative *Staphylococcus* probes were further tested against DNAs from sets of related species, as follows.

Six isolates of *S. agalactiae* and four isolates of *S. pneumoniae* were tested against the *S. agalactiae* and *S. pneumoniae* probes as described above, as were 13 isolates of a total of 12 other streptococcal species (Table 5). The *S. agalactiae* probe KG0001 hybridized to DNAs from *Streptococcus* group G, *S. pyogenes*, and *S. dysgalactiae*. These species, along with *S. agalactiae*, comprise the "pyogenic, hemolytic" group of streptococci (13). KG0001 also hybridized to DNA from *S. salivarius*, an oral *Streptococcus* sp. The *S. pneumoniae* probe RDR462 detected *S. mitis* in addition to *S. pneumoniae*. *S. mitis* is an oral *Streptococcus* sp. (13).

The *Neisseria* probe COR28 was tested against other *N. meningitidis* serotypes and multiple isolates of closely related species (Table 5). The probe hybridized to DNAs from *N. meningitidis* serotypes A, B, C, Y, and W135, *N. gonorrhoeae* subsp. *kochii*, and *N. gonorrhoeae*. The *Neisseria* probe did not hybridize to DNAs from any of the following closely related species: *N. cinerea*, *N. sicca*, *N. polysaccharea*, and *Eikenella corrodens*.

The *E. coli*-enteric bacterium probe RDR140 was tested against a panel of 45 gram-positive and 33 gram-negative bacteria, including 9 different species of enteric bacteria (Table 1): *E. coli*, *Citrobacter freundii*, *Edwardsiella tarda*, *Enterobacter cloacae*, *Proteus mirabilis*, *Providencia stuartii*, *Rahnella aquatilis*, *Serratia marcescens*, and *Yersinia enterocolitica*. The probe hybridized to all of the DNAs from the enteric species and to none of the DNAs from the nonenteric species.

In addition to the *C. genitalium*, *C. pseudotuberculosis*, and *C. xerosis* strains mentioned above and listed in Table 4, the *Corynebacterium* probe RDR510 was tested against an additional *Corynebacterium* species, *C. jeikeium*, and gave a positive signal. The *Propionibacterium* probe, tested above with *P. acnes*, was also tested against *P. avidum*, *P. lymphophilum*, and *P. granulosum* and gave positive signals. In addition to the *B. subtilis*, *B. cereus*, and *B. amylolique-*

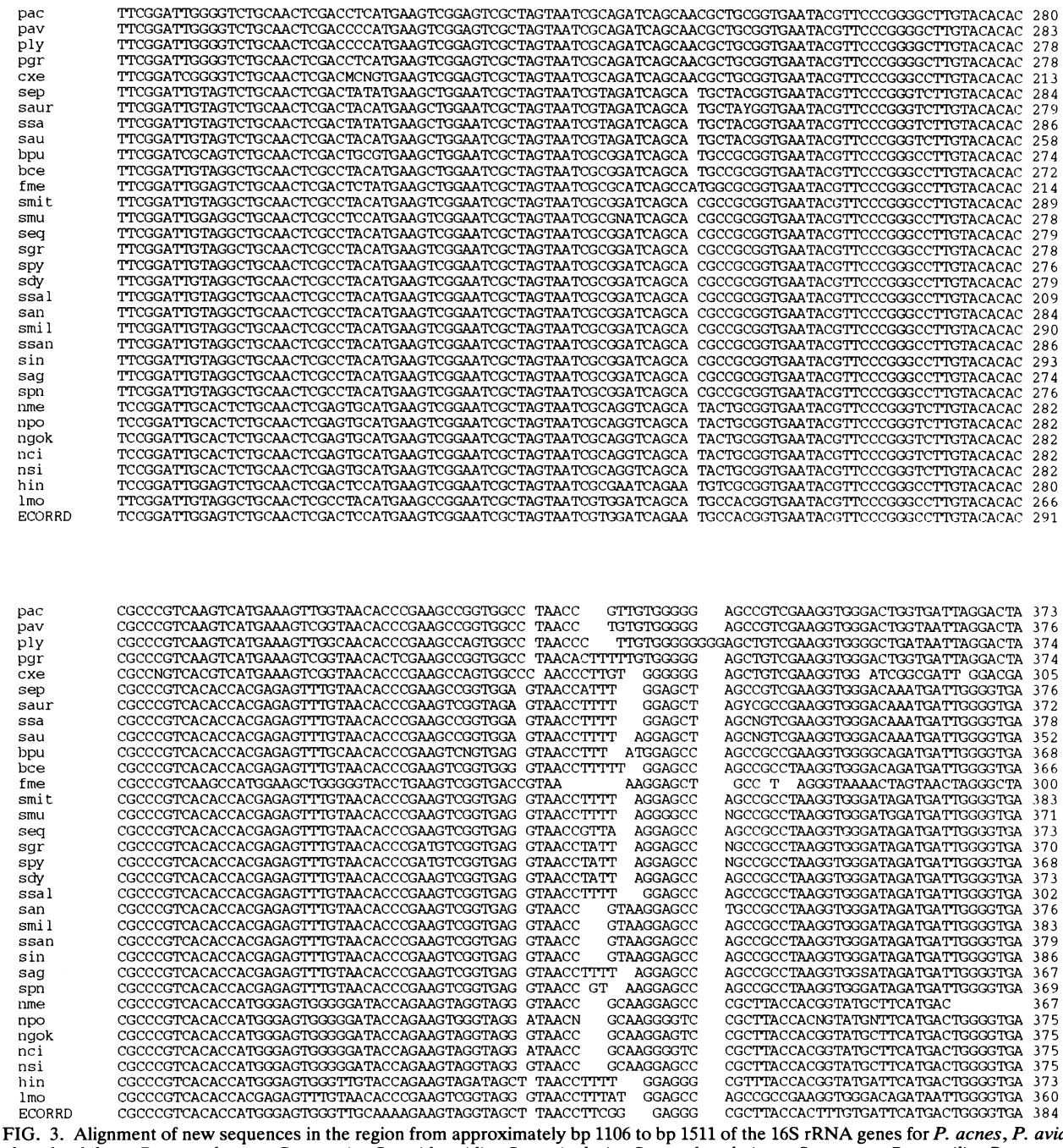

FIG. 3. Alignment of new sequences in the region from approximately bp 1106 to bp 1511 of the 16S rRNA genes for *P. acnes*, *P. avidum*, *P. lymphophilum*, *P. granulosum*, *C. xerosis*, *S. epidermidis*, *S. auricularis*, *S. saccharolyticus*, *S. aureus*, *B. pumilis*, *B. cereus*, *F. meningosepticum*, *S. mitis*, *S. mutans*, *S. equi*, *Streptococcus* group G, *S. pyogenes*, *S. dysgalactiae*, *S. salivarius*, *S. anginosus*, *S. milleri*, *S. sanguis*, *S. intermedius*, *S. agalactiae*, *S. pneumoniae*, *N. meningitidis*, *N. polysaccharea*, *N. gonorrhoeae* subsp. *kochii*, *N. cinerea*, *N. sicca*, *H. influenzae*, and *L. monocytogenes* (lines 1 to 31, respectively). The *E. coli* sequence is aligned as a reference (line 32). Nucleotide symbols are used as recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

faciens strains tested above, *B. pumilis* and *B. brevis* also tested positive with the *Bacillus* probe RDR502.

The *Staphylococcus* probes RDR325, RDR512, and RDR327 were tested against eight other *Staphylococcus* species—*S. auricularis*, *S. saccharolyticus*, *S. capitis* subsp. *capitis*, *S. cohnii* subsp. *cohnii*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, and *S. warneri* (Table 5). One of the coagulase-negative *Staphylococcus* probes, RDR325, hybridized to *S. haemolyticus* DNA in addition to *S. epidermidis* DNA. The other coagulase-negative *Staphylococcus* probe, RDR512, hybridized to *S. auricularis*, *S. saccharolyticus*, and *S. capitis* subsp. *capitis* DNAs. The coagulase-negative species DNAs which did not hybridize to either probe at 66°C were from *S. cohnii* subsp. *cohnii*, *S. hominis*, *S. saprophyticus*, and *S. warneri*. The *S. aureus* probe RDR327 hybridized to *S. aureus* and *S. cohnii* subsp. *cohnii*

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pac                    CTGTTGCCAGCACGTTATGGTGGGGACTCAGTGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCA 84
pav                  TCACTGTTGCCAGCACGTTATGGTGGGGACTCAGTGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCA 87
ply                     GTTGCCAGCAAGTTATGTTGGGGACTCGTTAGAGACCGCCGAGGTCAACTCGGAGGAAGGTGAGGACGACGTCAAGTCATCA 82
pgr                TCCACTGTTGCCAGCAA TTCGGKGGGGGACTCAGTGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCA 82
cxe                                                                                    AT ACGTCAAATCATCA 16
sep              CTTAAGCTTAGTTGCCATC A TTAAGTTGGGCACTCTAAGTTGACNGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 89
saur                CTTAGTTGCCATC A TTCAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGRGATGACGTCAAATCATCA 84
ssa              CCTTAAGCTTAGTTGCCATC A TTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 91
sau                                  GGGCACTCTAAGTTGACNGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 63
bpu                     GTTGCCAGC A TTCAGTTGGGCACTCTAAGGTGACNGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 79
bce                       GCCATC A TTAAGTTGGGCACTCTAAGGTGACNGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 77
fme                                                                                    TGACGTCNAATCATCA 18
smit          AACCCTTATTGTTAGTTGCCATC A TTCAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 94
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ssal                                                                                   ATGACGTCAANTCATCA 16
san                TTATNGTTAGTTGCCATC A TTAAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 89
smil          CAACCCTTATTGTTAGTTGCCATC A TTGAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 95
ssan             CCC TATTGTTAGTTGCCATC A TTCAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 91
sin           GCGCAACCCTTATTGTTAGTTGCCATC A TTCAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 98
sag                     TTGCCATC A TTAAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 79
spn                     AGTTGCCATC A TTTAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCA 81
nme                GTCATTAGTTGCCATC A TTCAGTTGGGCACTCTAATGAGACTGCCGGTGACAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCA 87
npo                GTCATTAGTTGCCATC A TTCGGTNGGGCACTCTAATGAGACNGCCGGTGACNAGCCGGAGGAAGGTGGGGATGACNTCAAGTCCTCA 87
ngok               GTCATTAGTTGCCATC A TTCAGTTGGGCACTCTAATGAGACTGCCGGTGACAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCA 87
nci                GTCATTAGTTGCCATC A TTTAGTTGGGCACTCTAATGAGACTGCCGGTGACAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCA 87
nsi                GTCATTAGTTGCCATC A TTAAGTTGGGCACTCTAATGAGACTGCCGGTGACAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCA 87
hin                   CTTTGTTGCCAGCGACTT GGTCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA 85
lmo                              C A TTTAGTTGGGCACTCTAAAGTGACTGCCGGTG CAAGCCGGAGGAAGGTGGGGATGACGTCAAATCATCA 71
ECORRD        CAACCCTTATCCTTTGTTGCCAGCG GTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCA 96

pac          TGCCCCTTATGTCCAGGGC  TTCACGCATGCTACAATGGCTGGTACAGAGAGTGGCGA GC CTGTGAGGGTGAGCGAATCTCGGAAAGCCGGTCTCAG 180
pav          TGCCCCTTATGTCCAGGGC  TTCACGCATGCTACAATGGCCGGTACAAAGAGTTGCGA GC CTGTGAGGGTGAGCGAATCTCGGAAAGCCGGTCTCAG 183
ply          TGCCCCTTATGTCCAGGGC  TTCACGCATGCTACAATGGTCGGTACAGTCAGTTGCGA GC CTGTAAGGGTTAGCGAATCTGTAAAAGCCGGCCTCAG 178
pgr          TGCCCCTTATGTCCAGGGC  TTCACGCATGCTACAATGGCCGGTACAGTGAGTTGCGA CATC GTAAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAG 178
cxe          TGCCCCTTATGTCCAGGGCATTTCACACATGCTACAATGGTCGGTACAGTGGGTTGCGATG CCG TGAGGTGGAGCTAATCCCTGAAAGCCGGTCTCAG 114
sep          TGCCCCTTATGATTTGGGC  TACACACGTGCTACAATGGACAATACAAAGGGYAGCGAA ACCGC GAGGTCAAGCAAATCCCATAAAGTTGTTCTCAG 185
saur         TGCCCCTTATGATTTGGGC  TACACACGTGCTACAATGGACAATACAAAGGGCAGCGAA ACCGC GAGGTCAAGCAAATCCCATAAAGTTGTTCTCAG 180
ssa          TGCCCCTTATGATTTGGGC  TACACACGTGCTACAATGGACAATACAAAGGGCAGCGAA ACCGC GAGGTCAAGCAAACCCCATAAAGTTGTTCTCAG 187
sau          TGCCCCTTATGATTTGGGC  TACACACGTGCTACAATGGACAATACAAAGGGCAGCGAA ACCGC GAGGTCAAGCAAATCCCATAAAGTTGTTCTCAG 159
bpu          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGACAGAACAAAGGGCTGC AAGACCGC AAGGTTTAGCCAATCCCATAAATCTGTTCTCAG 175
bce          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGACGGTACAAAGAGCTGC AAGACCGC GAGGTGGAGCTAATCTCATAAAACCGTTCTCAG 173
fme          CGGCCCTTACGTCTTGGGC CNACACACGTGCTACAATGGCCGGTACAGAGGGCAGCTA CCTAGC GATAGGATGCAAATCTC GAAAGCCGGTCTCAG 114
smit         TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGT CGGTGACGGCAAGCTAATCTCTTAAAGCCAGTCTCAG 190
smu          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTCGGTACAACGAGTTGC GAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCGATCTCAG 180
seq          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCAATCTCAG 180
sgr          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAG 179
spy          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAG 177
sdy          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAG 180
ssal         TGCCC TTATGACCTGG C  TACACACGTGCTACAATGGTTGGTACAACGAGTTGCGA GT CGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAG 110
san          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCAGTCTCAG 185
smil         TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCAGTCTCAG 191
ssan         TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCAGTCTCAG 187
sin          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTGAAAGCCAGTCTCAG 194
sag          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGTTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAG 175
spn          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGCTGGTACAACGAGTCGC AAGC CGGTGACGGCAAGCTAATCTCTTAAAGCCAGTCTCAG 177
nme          TGGCCCTTATGACCAGGGC  TTCACACGTCATACAATGGTCGGTACAGAGGGTAGCCAAGC CG CGAGGCGGAGCCAATCTCACAAAACCGATCGTAG 183
npo          TGGCCCTTATGACCAGGGC  TTCACACGTCATACAATGGTCGGTACAGAGGGTAGCCAAGC CG CGAGGTGGAGCCAATCTCACAAAACCGATNGTAG 183
ngok         TGGCCCTTATGACCAGGGC  TTCACACGTCATACAATGGTCGGTACAGAGGGTAGCCAAGC CG CGAGGCGGAGCCAATCTCACAAAACCGATCGTAG 183
nci          TGGCCCTTATGACCAGGGC  TTCACACGTCATACAATGGTCGGTACAGAGGGTAGCCAAGC CG CGAGGTGGAGCCAATCTCACAAAACCGATCGTAG 183
nsi          TGGCCCTTATGACCAGGGC  TTCACACGTCATACAATGGTCGGTACAGAGGGTAGCCAAGC CG CGAGGTGGAGCCAATCTCACAAAACCGATCGTAG 183
hin          TGGCCCTTACGAGTAGGGC  TACACACGTGCTACAATGGCGTATACAGAGGGAAGCGAAGCT GC GAGGTGGAGCGAATCTCATAAAGTACGTCTAAG 181
lmo          TGCCCCTTATGACCTGGGC  TACACACGTGCTACAATGGATAGTACAAAGGGTCGCGAAGC CGC GAGGTGGAGCTAATCCCATAAAACTATTCTCAG 167
ECORRD       TGGCCCTTACGACCAGGGC  TACACACGTGCTACAATGGCGCATACAAAGAGAAGCGA CCTCGC GAGAGCAAGCGGACCTCATAAAGTGCGTCGTAG 192
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FIG. 3—*Continued.*

DNAs but to none of the DNAs from the other coagulase-negative staphylococci.

DISCUSSION

The PCR primers described were designed to detect bacteria present in normally sterile body fluids, such as blood and CSF. Since a variety of bacterial species can cause septicemia and meningitis, the primers were designed to amplify DNAs from phylogenetically divergent bacteria by targeting conserved regions of the 16S rRNA gene. As the amplifications with the panel of 176 different bacterial isolates in this study indicate, primers RW01, DG74, and RDR080 are capable of amplifying DNAs from a broad range of bacteria, producing PCR products for almost all of the species tested.

The routine use of universal bacterial primers requires caution in the setup and amplification procedures used. In addition to precautions which are applicable to any low-copy-number DNA amplifications (use of positive-displacement pipettes and separation of areas for PCR setup and analysis of amplified products) (20), other precautions were necessary with the use of the universal bacterial primers. In particular, it was necessary to determine the maximum

pac	AGTCGTAACAAGGT	387
pav	AGTCGTAAC	385
ply	AGTCGTAACAAGGT	388
pgr	AGTCGT	380
cxe	NGTCGTA	312
sep	NGTCGTAACAAGGTAGCCG	394
saur	NGTCGTAACAAGGTAGCCG	390
ssa	NGTCGTAACAAGGTAGCCG	396
sau	GTCGTAACAAGGTA	366
bpu	NGTCGTAACAAGGT	381
bce	TGTCGTAAC	374
fme	AGTCGTA	307
smit	AGTCGTAACAAGG	396
smu	AGTCGTAACAAGGTAGCCG	390
seq	TGTCGTAACAAGGTAGCCG	391
sgr	NGTCGTAACAAGGTAGCCG	388
spy	NGTCGTAACAAGGTAGCCG	386
sdy	NGTCGTAACAAGGTAGCCG	391
ssal	AGTCGTAACAAGGTAG	318
san	TGTCGTAACAAGGTAGCCG	394
smil	TGTCGTAACAAGGTAGCCG	401
ssan	AGTCGTAACAAGGTAGCCG	398
sin	AGTCGTAACAAGGTAGCCG	405
sag	CGTCGTAACAAGGTAGCC	385
spn	AGTCGTAACAAGGTAGC	386
nme		
npo	NGTCGTAACAAGGTNGC	
ngok		
nci		
nsi	NGTCGTAACAAGGT	388
hin	AGTCGTAACAAGGTAACC	391
lmo	AGTCGTAACAAGGTAGC	377
ECORRD	AGTCGTAACAAGGTAACCG	403

FIG. 3—*Continued.*

number of cycles of amplification to use without obtaining a DNA product in no-DNA controls. The DNA product observed in no-DNA controls was most likely due to the presence of bacterial DNA in *Taq* polymerase preparations or other PCR components (34). With the use of 25 cycles, no contaminating DNA was visible on ethidium bromide-stained gels or after probe hybridization. Higher cycle numbers can be used when *Taq* polymerase and other PCR components have been treated to reduce the amount of contaminating DNA.

There were differences in the efficiency of amplification of the strains examined. Some of the strains were predicted to have mismatches with the 3′ terminus of primer RW01 and were amplified better with RDR080 (*C. fetus*, *C. jejuni*, *P. lymphophilum*, and *M. luteus*). Two strains gave amplification results which were discrepant relative to published sequence information. *A. israelii* gave a higher PCR product yield with RDR080 than with RW01, even though it was predicted to have a perfect match with the 3′ end of RW01. *Vibrio parahaemolyticus* was predicted to have a perfect match with the 3′ end of RDR080 but gave a PCR product yield equal to or lower than that obtained with RW01.

Some of the species whose DNAs were not amplified as efficiently are known to have high overall G+C contents; these include *Actinomyces*, *Mycobacterium*, *Mycoplasma*, and *Micrococcus* species. A number of modifications of the amplification procedure described above were tried to overcome the effect of a high G+C content. Modifications such as raising the denaturation temperature, lengthening the time of denaturation and annealing, and adding cosolvents, such as formamide and glycerol, did not substantially alter the level of amplification for *M. tuberculosis* DNA (unpublished data). In preliminary experiments with DNA polymerase from *Thermococcus litoralis*, a higher level of amplification of *M. lysodeikticus* DNA was observed. However, a higher level of background amplification (amplification in no-DNA controls) was also seen. Since other amplification systems, such as those which are specific for *M. tuberculosis*, display higher sensitivity, these observations are most likely due to the nature of the particular primers and/or target gene used.

Among the broad-range probes tested, the gram-positive, two gram-negative, and *Bacteroides* probes were quite specific, differentiating correctly 100 of the 102 different species tested. *D. radiopugnans* hybridized with neither the gram-positive nor the gram-negative probes. This species is classified as gram positive, although studies have shown it to be more similar to gram-negative bacteria than to gram-positive bacteria, with respect to its having an outer membrane and to the characteristics of its fatty acid profile (29). *G. vaginalis* did not hybridize with the gram-positive or gram-negative probes either. This organism has characteristics of the gram-positive cell wall but stains gram negative or gram variable (32).

The series of probes for the identification of bacteria in CSF was designed on the basis of nucleotide sequence information obtained during this investigation and on sequence information available from GenBank and EMBL. Hybridization testing of the probes indicated that there was some cross-reactivity for each probe against related species. Possible cross-reactions for each of the probes are noted specifically below.

By Southern blot hybridization, each of the 12 meningitis and contaminant probes was tested against the seven major bacterial species causing meningitis (*H. influenzae*, *S. pneumoniae*, *S. agalactiae*, *N. meningitidis*, *E. coli*, *L. monocytogenes*, and *S. aureus*), with each of the species being represented by two to six different isolates. For *L. monocytogenes*, the five isolates tested were each of a different serotype; for *N. meningitidis*, two isolates of serotype A were tested. In each case, the probes gave the correct pattern of hybridization, with the exception of *N. gonorrhoeae*, as noted earlier. The expected hybridization results were also obtained when the 12 probes were tested against the four contaminant genera, with one to six isolates or species of each genus being tested (three *Bacillus* species, one *Propionibacterium* species, three *Corynebacterium* spe-

cies, and six isolates of *S. epidermidis*). Additional, more limited hybridization testing of specific probes was done with 12 *Streptococcus* species, four additional serotypes of *N. meningitidis*, 5 *Neisseria* species, 8 enteric bacteria, 1 *Corynebacterium* species, 8 *Staphylococcus* species, 2 *Bacillus* species, and 3 *Propionibacterium* species.

In addition to the hybridization testing, the probe sequences were compared with available sequence information for both the species to be detected and closely related species. Some of the sequence information became available after the probes were designed. Criteria used to predict the reactions were positive if there was a perfect match between probe and target organism and negative if there were more than two mismatches within the central 10 bases of the probe. One-base mismatches were predicted on the basis of hybridization results, if available, for the same species or species predicted to have the same sequence in the region of the probe. The combination of the hybridization results and the sequence comparisons for each probe are summarized as follows.

The *Haemophilus* probe sequence was compared with the 16S gene sequences of 22 *Haemophilus* species, 16 *Pasteurella* species, and 15 *Actinobacillus* species available in the GenBank data base (accession numbers M75035 to M75084 and M35017 to M35019) (12). This analysis indicated that, in the region of the probe, there is no difference among the *Haemophilus*, *Actinobacillus*, and *Pasteurella* species analyzed. Hence, this probe is more accurately termed a *Pasteurellaceae* genus probe. None of the *Haemophilus* species other than *H. influenzae* or any *Actinobacillus* or *Pastereurella* species, however, are commonly found in CSF (16, 32).

The *S. pneumoniae* and *S. agalactiae* probe sequences were compared with the 16S rRNA sequences in the GenBank data base for 32 streptococcal species (2); the sequence data and hybridization results obtained in this study were used to predict the reactions for these sequences. The *S. pneumoniae* probe is predicted to hybridize fully to PCR products from *S. pneumoniae*, *S. oralis*, and *S. parasanguis* and partially to PCR products from *S. anginosus*, *S. intermedius*, and *S. sanguis*. Oral streptococci, such as *S. oralis*, *S. sanguis*, *S. anginosus*, and *S. intermedius*, can be found in the CSF of patients, especially that of neutropenic patients. The *S. agalactiae* probe is predicted to hybridize to *S. agalactiae* DNA and to DNAs of other members of the pyogenic, hemolytic group of streptococci, including *S. pyogenes* and *S. dysgalactiae*, and to other streptococci, including *S. salivarius*, *S. alactolyticus*, *S. bovis*, and *S. equinus*. *S. pyogenes* is only occasionally found in CSF (13).

The *N. meningitidis* probe COR28 is predicted not to react with DNAs from other members of the family *Neisseriaceae* or the beta group of the *Proteobacteria* (11), except for *N. gonorrhoeae* (X07714); these include *Kingella denitrificans* (M22516), *K. indologenes* (M35015), *K. kingae* (M22517), *N. denitrificans* (M35020), *A. faecalis* (M22508), *Chromobacterium violaceum* (M22510), *C. fluviatile* (M22511), *E. corrodens* (M22512, M22513, and M22514), *F. lutescens* (M59156), and *Vitreoscilla stercoraria* (M22519). The predictions for *E. corrodens* and *N. gonorrhoeae* are consistent with the results observed experimentally.

Among the close relatives of *N. meningitidis* tested, the *Neisseria* probe cross-reacts only with *N. gonorrhoeae* and *N. gonorrhoeae* subsp. *kochii* DNAs. *N. gonorrhoeae* subsp. *kochii* has been shown to exhibit biochemical and serologic characteristics of both *N. gonorrhoeae* and *N. meningitidis* (18). It has been isolated from patients with conjunctivitis in rural Egypt and also from men with urethritis in Alexandria, Egypt (26). Neither of these species is commonly found in CSF specimens (28).

The *E. coli*-enteric bacterium probe RDR140 was screened against the sequences of the following members of the family *Enterobacteriaceae*: *P. vulgaris* (X07652 and J01874), *C. freundii* (M59291), *Erwinia carotovora* (M59149), *Hafnia alvei* (M59155), *S. marcescens* (M59160), and *Y. enterocolitica* (M59292); it was also screened against the sequences *Serratia* species (10) *S. marcescens*, *S. ficaria*, *S. entomophila*, *S. liquefaciens*, *S. plymuthica*, *S. proteamaculans*, *S. grimesii*, *S. rubidaea*, *S. odorifera*, and *S. fonticola* and is predicted to hybridize to all of these DNAs. The predicted results for *C. freundii*, *S. marcescens*, and *Y. enterocolitica* are consistent with the positive results observed experimentally. The combination of hybridization results and sequence analyses available to date indicates that RDR140 is specific for enteric species. Some less common enteric species, however, have not been tested.

The *L. monocytogenes* probe RDR230 is a perfect match to the *L. monocytogenes* sequences in the GenBank data base (M58822 and X56153). This probe will probably not react with *L. grayi* or *L. murrayi* DNA (8). It contains one central G · T mismatch with the *L. seeligeri*, *L. innocua*, *L. ivanovii*, and *L. welshimeri* sequences and may or may not hybridize with DNAs from these species, depending on the stringency of the hybridization and washing conditions used (8). The *L. monocytogenes* probe appears to be relatively broad in range, in that it hybridizes with DNAs from five of the serotypes commonly found in clinical specimens, including the three serotypes (1/2a, 1/2b, and 4b) that represent 90% of human and animal isolates. The other *Listeria* species which it may detect (*L. seeligeri*, *L. innocua*, *L. ivanovii*, and *L. welshimeri*) are not commonly found in CSF (3).

The *Bacillus* probe was compared with the published sequences of 55 *Bacillus* species, corresponding to accession numbers X55059 to X55063, X57304 to X57309, and X60601 to X60645. Among *Bacillus* species known to be widespread in the environment (7), this probe is predicted to hybridize to DNAs from *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *B. macerans*, *B. lautus*, *B. circulans*, *B. brevis*, *B. aneurolyticus*, *B. pumilis*, *B. thuringiensis*, *B. polymyxa*, *B. badius*, *B. pantothenticus*, *B. licheniformis*, *B. coagulans*, and *B. sphaericus*. The *B. pasteurii* and *B. lentus* sequences contained at least two mismatches, indicating that they probably would not hybridize to the probe. The *Bacillus* probe is therefore expected to hybridize to DNAs from most of the *Bacillus* species commonly present in the environment.

Screening of the data bases with the *Bacillus* probe sequence indicated that this probe is predicted to hybridize to DNAs of some gram-negative bacteria, such as *Rickettsia* spp. (M21798, M21293, and M20499), *Brucella abortus* (X13695), and *Pseudomonas cepacia* (M22518). These gram-negative species are not commonly found in CSF. The specificity of the *Bacillus* probe could be augmented by use of the gram-negative probes DL04 and RDR278 to differentiate gram-positive *Bacillus* species from cross-reacting gram-negative species.

The *Corynebacterium* probe RDR510 will react with *C. variabilis* (X53185) and *C. renale* (M29553) DNAs. This probe has not been tested against the nontyped coryneform strains which are known to colonize the skin (33). Data base screening indicated that this probe is predicted to hybridize to DNAs from the following gram-negative species: *Mycoplasma* species (M96660, M24579, M24580, M24658, and M24661), *K. indologenes* (M35015), *Cardiobacterium hominis* (M35014), *Acinetobacter* species (M34139 and M22351),

TABLE 4. Hybridization results with CSF probes

Organism	Strain	Result[a] with the following probe:						
		RDR125 (*H. influenzae*)	RDR462 (*S. pneumoniae*)	KG0001 (*S. agalactiae*)	COR28 (neisseriae)	RDR140 (*E. coli*)	RDR230 (*L. monocytogenes*)	RDR502B (bacillus)
Haemophilus influenzae	ATCC 33391	+	−	−	−	−	−	−
	2423	+	−	−	−	−	−	−
	503-1156	+	−	−	−	−	−	−
	503-1148	+	−	−	−	−	−	−
	503-1155	+	−	−	−	−	−	−
	503-1154	+	−	−	−	−	−	−
Streptococcus pneumoniae	ATCC 33400	−	+	−	−	−	−	−
	ATCC 6303	−	+	−	−	−	−	−
	4366	−	+	−	−	−	−	−
	4471	−	+	−	−	−	−	−
S. salivarius	ATCC 13419	−	−	+	−	−	−	−
	ATCC 7073	−	−	+	−	−	−	−
S. agalactiae	ATCC 13813	−	−	+	−	−	−	−
	4352	−	−	+	−	−	−	−
	4353	−	−	+	−	−	−	−
	4354	−	−	+	−	−	−	−
	4355	−	−	+	−	−	−	−
	4356	−	−	+	ND	−	−	−
Neisseria meningitidis	CMCC 2801	−	−	−	+	−	−	−
	ATCC 13077	−	−	−	+	−	−	−
N. gonorrhoeae	CMCC 2783	−	−	−	+	−	−	−
	ATCC 19424	−	−	−	+	−	−	−
	31917	−	−	−	+	−	−	−
	31959	−	−	−	+	−	−	−
	32171	−	−	−	+	−	−	−
	32213	−	−	−	+	−	−	−
Escherichia coli	Strain B	−	−	−	−	+	−	−
	ATCC 11775	−	−	−	−	+	−	−
	9	−	−	−	−	+	−	−
	P3478	−	−	−	−	+	−	−
	2889	−	−	−	−	+	−	−
	340	−	−	−	−	+	−	−
Listeria monocytogenes	ATCC 15313	−	−	−	−	−	+	−
	G0282	−	−	−	−	−	+	−
	G0288	−	−	−	−	−	+	−
	F9784	−	−	−	−	−	+	−
	G0278	−	−	−	−	−	+	−
	F9841	−	−	−	−	−	+	−
Bacillus subtilis	BD224	−	−	−	−	−	−	+
	ATCC 6051	−	−	−	−	−	−	+
	558	−	−	−	−	−	−	+
B. cereus	ATCC 11778	−	−	−	−	−	−	+
B. amyloliquefaciens	H	−	−	−	−	−	−	+
Corynebacterium genitalium	ATCC 33030	−	−	−	−	−	−	−
C. pseudotuberculosis	ATCC 19410	−	−	−	−	−	−	−
C. xerosis	ATCC 373	−	−	−	−	−	−	−
Propionibacterium acnes	ATCC 6919	−	−	−	−	−	−	−
Staphylococcus epidermidis	ATCC 12228	−	−	−	−	−	−	−
	ATCC 14990	−	−	−	−	−	−	−
	4233	−	−	−	−	−	−	−
	4234	−	−	−	−	−	−	−
	4235	−	−	−	−	−	−	−
	4236	−	−	−	−	−	−	−
S. aureus	ATCC 33589	−	−	−	−	−	−	−
	ATCC 25923	−	−	−	−	−	−	−
	4241	−	−	−	−	−	−	−
	4247	−	−	−	−	−	−	−
	4248	−	−	−	−	−	−	−
	4249	−	−	−	−	−	−	−
Flavobacterium meningosepticum	ATCC 13253	−	−	−	−	−	−	−

[a] ND, not done.

TABLE 4—*Continued*

Result[a] with the following probe:				
RDR510 (coryne-bacteria)	RDR514 (propioni-bacteria)	RDR325 (*S. epidermidis*)	RDR512 (coagulase-negative staphylococci)	RDR327 (*S. aureus*)
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and *P. aeruginosa* (M34133 and X06684). *P. aeruginosa* can be found in CSF specimens as a cause of meningitis. As with the *Bacillus* probe, the gram-negative probes can be used to differentiate gram-positive *Corynebacterium* species from cross-reacting gram-negative species.

The *Propionibacterium* probe was compared with the following sequences: *P. acnes* (X53218), *P. freudenreichii* (X53217), *P. jensenii* (X53219), and *P. propionicus* (X53216); however, it is not possible to predict the hybridization reactions, since there are two N's in the probe region for each of these species. (The *P. acnes* sequence obtained experimentally and used to design the *Propionibacterium* probe was completely readable in the probe region.) The RDR514 probe will not react with *P. acidipropionici* or *P. thoenii* DNA. *P. freudenreichii*, *P. jensenii*, *P. propionicus*, *P. acidipropionici*, and *P. thoenii* are found in cheese and dairy products. The *Propionibacterium* species which are typically found on human skin, in addition to *P. acnes*, and *P. avidum* and *P. granulosum*, both of which will react with the *Propionibacterium* probe (9).

There was no existing sequence information against which to compare the *Staphylococcus* probes. The hybridization results indicated that the *S. aureus* probe will hybridize to *S. aureus* and *S. cohnii* DNAs. *S. cohnii* is occasionally isolated from clinical samples (17). The two coagulase-negative *Staphylococcus* probes together hybridized to DNAs from five of nine coagulase-negative *Staphylococcus* species tested, the exceptions being *S. cohnii*, *S. hominis*, *S. saprophyticus*, and *S. warneri*. Lowering the hybridization temperatures in an attempt to increase the range of specificity of these probes did not allow the detection of additional species. All nine of the coagulase-negative species tested are commonly found on the skin and may also cause opportunistic infections. Hence, the coagulase-negative *Staphylococcus* probes RDR325 and RDR512 are not broad enough in range for use with clinical samples. With the exception of these coagulase-negative *Staphylococcus* probes and within the set of bacteria typically found in human CSF, the specificity of the CSF pathogen and contaminant probes described above is predicted to be sufficient to provide correct identification.

The universal primers used in this study provide a sensitivity in ethidium bromide-stained gels of 10 gene copies of *E. coli* DNA, which corresponds roughly to three *E. coli* cells, under conditions appropriate for higher cycle numbers. In one report, it was estimated that 85% of CSF samples contain over 10^3 CFU/ml (21). A clinical PCR assay based on these primers may have sufficient sensitivity to allow direct detection of bacteria in CSF without an intermediate culturing step. Culturing would still be required, however, for the determination of antibiotic susceptibility of any bacteria detected.

The meningitis probes described in this study identify the most frequent causes of bacterial meningitis. For meningitis pathogens which are not targeted with more specific probes, the broad-range probes described here, the universal bacterial, gram-negative, gram-positive, and *Bacteroides-Flavobacterium* probes, will be useful. This group of organisms could include (i) species which are known to cause meningitis or septicemia but at a low frequency (such as *P. aeruginosa*, *Legionella* species, and *Enterococcus* species) and (ii) novel species which are not yet characterized. Bacteria in the latter category may be undetectable by conventional culture methods because of fastidious growth characteristics or a low level of bacteria in the sample. The hybridization results presented above for the novel strains

TABLE 5. Additional hybridization testing of CSF probes

Additional bacterial species tested	Result with the following probe:					
	KG0001 (*S. agalactiae*)	RDR462 (*S. pneumoniae*)	COR28 (*N. meningitidis*)	RDR325 (coagulase-negative staphylococci)	RDR512 (coagulase-negative staphylococci)	RDR327 (*S. aureus*)
Streptococcus equi	−	−				
Streptococcus group G	+	−				
S. pyogenes	+	−				
S. dysgalactiae	+	−				
S. salivarius	+	−				
S. anginosus	−	−				
S. milleri	−	−				
S. mitis	−	+				
S. mutans	−	−				
S. sanguis	−	−				
S. intermedius	−	−				
Neisseria meningitidis						
Serotype A			+			
Serotype B			+			
Serotype C			+			
Serotype Y			+			
Serotype W135			+			
N. gonorrhoeae			+			
N. gonorrhoeae subsp. *kochii*						
NRL 32895			+			
NRL 32896			+			
NRL 31291			+			
NRL 31292			+			
NRL 31294			+			
CDC 10,046			+			
N. cinerea						
CDC 10,050			−			
CDC 10,051			−			
CDC 10,052			−			
CDC 10,053			−			
CDC 10,054			−			
N. sicca			−			
N. polysaccharea						
ATCC 43768			−			
CDC 10,049			−			
CDC 10,048			−			
CDC 10,047			−			
Eikenella corrodens			−			
Staphylococcus auricularis				−	+	−
S. saccharolyticus				−	+	−
S. capitis subsp. *capitis*				−	+	−
S. cohnii subsp. *cohnii*				−	−	+
S. haemolyticus				+	−	−
S. hominis				−	−	−
S. saprophyticus				−	−	−
S. warneri				−	−	−

indicate that it is possible to use the broad-range probes to obtain a reliable preliminary classification of new pathogens.

In a clinical setting, the use of a panel of probes is suited to a detection format in which the probes, instead of the PCR product, are immobilized on a solid support, such as a reverse dot blot format or a microwell plate format (22, 36). This format would enable the use a single CSF sample to obtain multiple probe hybridization results. The PCR primers and panel of probes described here can form the basis of a more rapid and sensitive means of detecting bacteria in clinical samples.

ACKNOWLEDGMENTS

We thank Deborah Dodge, Roche Molecular Systems, for advice on growing the bacterial cultures; Shi-Da Lu, Roche Molecular Systems, for DNA sequence information used to design the *E. coli*-enteric bacterium probe; and Howard Faden and Dan Lucey for bacterial strains. PCR primer DG74 was designed by David Gelfand, Roche Molecular Systems; PL06 was designed by Phoebe Landry, Roche Molecular Systems.

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