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# Species-Specific Oligodeoxynucleotide Probes for the Identification of Periodontal Bacteria

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**Oligodeoxynucleotide probes were developed for identification of the periodontal bacteria *Actinobacillus actinomycetemcomitans*, *Bacteroides gingivalis*, *B. intermedius* types I and II, *B. forsythus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Haemophilus aphrophilus*, *Streptococcus intermedius*, and *Wolinella recta*. Probes were designed by sequencing the 16S rRNA for each bacterium, identifying hypervariable regions, and chemically synthesizing species-specific probes. These probes were specific when tested against a panel of nucleic acids from closely related bacteria.**

Substantial evidence demonstrating the role of plaque bacteria in the etiology of periodontal diseases has been gathered (7, 16). Implication of specific microbes in disease has been based on their presence and relative numbers in healthy versus diseased subgingival sites. Traditionally, this correlation has been hampered by the time-consuming and tedious processes involved in using phenotypic markers for identification of the numerous aerobic and anaerobic species found in the oral microbiota.

More recently, identification of bacteria has been done on the basis of genomic similarities. One approach has been the use of whole-cell DNA probes which can identify pure isolates of *Bacteroides* spp. and other bacterial species (4, 10, 11, 18, 21–23). Although these methods are more convenient and cost effective than conventional techniques, they are unsuitable for use with highly mixed samples, such as subgingival plaque, because of the genomic homologies between closely related species (9, 23).

An alternative and potentially more specific approach is based on the sequence divergence of rRNAs (3, 5, 12, 17, 25). The nucleotide sequences of rRNAs have been largely conserved through evolution but contain regions which are divergent. Many of these changes exist in blocks, allowing synthesis of probes which can easily distinguish between very closely related species (2, 6).

We report here the design and testing of a panel of oligodeoxynucleotide probes for 10 species of oral bacteria, 8 of which have been implicated in periodontal disease. These bacteria are *Actinomyces actinomycetemcomitans*, *Bacteroides gingivalis*, *B. intermedius* types I (ATCC 25611 homology group) and II (ATCC 33563 homology group), *Fusobacterium nucleatum*, *Eikenella corrodens*, *Wolinella recta*, *B. forsythus*, *Streptococcus intermedius*, and *Haemophilus aphrophilus*. The oligodeoxynucleotide probes were designed to target hypervariable regions of 16S rRNA. The analysis presented here demonstrates that a very high degree of specificity is possible with this approach. Furthermore, direct detection of bacteria from plaque samples is possible, making these probes valuable tools for studies relating to the etiology of periodontal disease.

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## MATERIALS AND METHODS

**Bacterial cultures.** Bacteria were cultured in supplemental brain heart infusion broth (13). The reference strains listed in Table 1 were obtained from the American Type Culture Collection (ATCC), Rockville, Md., and were verified biochemically. *W. recta* oligodeoxynucleotide probes were also screened against *Campylobacter coli*, *C. fetus*, *C. faecalis*, *C. jejuni*, *C. laridis*, and *C. pylori* (NCTC 11638). The *Campylobacter* species were a generous contribution from M. Brondson (University of Washington, Seattle).

**Preparation of total nucleic acids from bacterial cultures and subgingival plaque samples.** Each bacterial culture ($10^9$ bacteria) or subgingival plaque sample (one curette scrape) was dispersed in 0.5 ml of 150 mM NaCl–20 mM Tris hydrochloride (pH 8.0)–10 mM EDTA–10 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid] and immediately stored at –80°C. After brief thawing, lysozyme (Sigma Chemical Co., St. Louis, Mo.) was added to 1 mg/ml and the samples were incubated for 15 min at 37°C. Sodium dodecyl sulfate was added to 1% (wt/vol), and pronase E (Sigma) was added to 1 mg/ml. After 30 min of incubation at 37°C, each sample was extracted with an equal volume (~0.5 ml) of phenol-chloroform-isoamyl alcohol (12:12:1), and the aqueous phase was adjusted to 2.5 M ammonium acetate. Two and one-half volumes of ethanol was added, and the samples were chilled at −20 or −80°C. Total nucleic acids were pelleted by centrifugation for 10 min at 16,000 × *g*, rinsed with ethanol, dried briefly, and suspended in 10 mM Tris hydrochloride (pH 8)–1 mM EDTA. The total nucleic acids were then used directly for slot blots or purified further by CsCl density gradient centrifugation to isolate genomic DNA (15).

**Sequencing of 16S rRNA and synthesis of oligodeoxynucleotide probes.** The sequencing protocol was a modification of the dideoxynucleotide-terminated chain elongation method (20) as adapted for rRNA templates (12). Universal sequencing primers were as previously described (12). The following two additional primers were designed and used for sequencing: 5′-CACGA(G/A)CTGACGACA(G/A)CCATGC-3′ and 5′-TACGG(A/C/G/T)ATACCTTGTTACGAC-3′. Sequences were compared with other 16S rRNA sequences (8), and oligodeoxynucleotide probes were designed for maximum species specificity.

Oligodeoxynucleotide probes were synthesized on an Ap-

TABLE 1. Specificity panel for analysis of oligodeoxynucleotide probes

| Species | ATCC Number[a] | Abbreviation |
|---|---|---|
| *A. actinomycetemcomitans* | 33384[T] | A.a. |
| *B. corporis* | 33547[T] | B.c. |
| *B. denticola* | 33185[T] | B.d. |
| *B. forsythus* | 33384[T] | B.f. |
| *B. gingivalis* | 33277[T] | B.g. |
| *B. intermedius* type 1 | 25611[T] | B.i.I |
| *B. intermedius* type 2 | 33563[T] | B.i.II |
| *B. loescheii* | 15930[T] | B.l. |
| *B. melaninogenicus* | 25845[T] | B.m. |
| *E. coli* | 33694[T] | E.c. |
| *E. corrodens* | 23834[T] | Ek.c. |
| *F. mortiferum* | 25557[T] | F.m. |
| *F. nucleatum* | 25586[T] | F.n. |
| *F. periodonticum* | 33693[T] | F.p. |
| *H. aphrophilus* | 33894[T] | H.a. |
| *H. paraphrophilus* | 29241[T] | H.p. |
| *H. segnis* | 33393[T] | H.s. |
| *P. micros* | 29241[T] | P.m. |
| *S. intermedius* | 27335[T] | S.i. |
| *W. recta* | 33210[T] | W.r. |
| *W. succinogenes* | 29543[T] | W.s. |
| *C. albicans* | 18804[T] | C.a. |

[a] The ATCC number is the number assigned by the ATCC. Type strains are indicated by T superscripts.

TABLE 2. Summary of the sequences and specificities of the oligodeoxynucleotide probes

| Species | Probe Name | Sequence[a] | Specificity |
|---|---|---|---|
| A.a. | Aa-2 | CTTCGGGCACYAGGGCTAAACCCC | A.a. |
| B.f. | Bf-2 | CGTATCTCATTTTATTCCCCTGTA | B.f. |
| | Bf-4 | CTGTAGAGCTTACACTATATCGCA | B.f. |
| B.g. | Bg-1 | CAATACTCGTATCGCCCGTTATTC | B.g. |
| | Bg-3 | CTGTGGAAGCTTGACGGTATATCG | B.g. |
| | Bg-4 | GTATAAAAGAAGTTTAMAAYCCTT | B.g. |
| | Bg-5 | CCGATGCTTATTCTTACGGTACAT | B.g. |
| | Bg-6 | CCTTAGGACAGTCTTCCTTCACGC | B.g. |
| | Bg-7 | ATTACCCTAGTGCGCCCCTTGCGG | B.g. |
| | Bg-8 | GGTTTTCACCATCAGTCATCTACA | B.g. |
| B.i.I | 1Bi-1 | GGTCCTTATTCGAAGGGTAAATGC | B.i.I |
| | 1Bi-2 | CCCTAGGYGCGCTCCTCGCGGTTA | B.i.I |
| | 1Bi-3 | CACGTGCCCCACTTTACTCCCCAA | B.i.I |
| | 1Bi-5 | GAGTCAACATCTCTGTATCCTGCG | B.i.I |
| | 1Bi-6 | TAGCCGCTAACGCCAGGCGCTAAC | B.i.I |
| B.i.II | 2Bi-1 | ATGAGGTACATGCAATGGCGCACA | B.i.II |
| | 2Bi-2 | CGTGCGCCAATTTATTCCCACATA | B.i.II |
| B.i.[b] | Bi-4 | TTGCCCTAGGTCGCTCCTCGCGGT | B.i.[b] |
| Ek.c. | Eikc-1 | TTAGGTACCGTCAGCAAAAAGTGG | Ek.c |
| | Eikc-2 | GCACTTCCCTTTTCTTCCCTAACA | Ek.c |
| | Eikc-3 | TACCGTGGCAAGCGGGCTCCTTGC | Ek.c |
| | Eikc-4 | GTACGCTACTAAGCAATCAAGTTG | Ek.c |
| F.n. | Fn-1 | GTCATCGTGCACACAGAATTGCTG | F.n.,F.m.,F.p. |
| | Fn-2 | GTTGGTACCGTCATTTTTTTCTTC | F.n., F.p. |
| | Fn-3 | AGGTTTCCCCGAAGGCACTGAAAC | F.n., F.p. |
| H.a. | Ha-2 | CTACGGGCACTAAGCTTAAAGCAC | H.a. |
| S.i. | Si-1 | GTACCGTCACAGTATGAACTTTCC | S.i. |
| | Si-2 | TTCTCACACTCGTTCTTCCTTAAC | S.i. |
| W.r. | Wr-1 | GTACCGTCATAATTCTTTCCCAAG | W.r. |
| | Wr-2 | GGACCATAACCGGTTTGGTATTTG | W.r. |
| | Wr-3 | GCATTACTGCCTCGACTAGCGAAG | W.r. |

[a] Y represents C or T. M represents A or C according to the nomenclature committee of the International Union of Biochemistry.
[b] B.i. represents *B. intermedius* types I and II.

plied Biosystems 380B synthesizer by beta-cyanoethyl phosphoramidite chemistry and purified by polyacrylamide gels or high-pressure liquid chromatographic methods (Applied BioSystems User Bulletin No. 13).

**Immobilization of nucleic acids to solid supports.** Genomic DNA was denatured in 0.3 M NaOH at 25°C for 10 min. An equal volume of 2 M ammonium acetate was added, and the sample was applied to Nytran membranes assembled in a slot blot apparatus (Schleicher & Schuell, Inc., Keene, N.H.). Total nucleic acids were denatured in 4.6 M formaldehyde–6× SSC (0.9 M NaCl, 90 mM sodium citrate) for 15 min at 60°C and immediately applied to Nytran membranes in a slot blot. After DNAs or total nucleic acids were immobilized, the membranes were baked for 1 h at 80°C and stored at room temperature.

**Labeling of probes and hybridizations.** Genomic probes were labeled with [$^{32}$P]dATP (ICN Pharmaceuticals Inc., Irvine, Calif.) by nick translation (15). Oligodeoxynucleotide probes were end labeled with [$^{32}$P]ATP (Du Pont Co., Wilmington, Del.) by using T4 polynucleotide kinase (15). All labeled probes were purified by Elutip-D chromatography (Schleicher & Schuell). The slot blots were hybridized with 1 to 5 ng of labeled probe per ml in 0.6 M NaCl–90 mM Tris hydrochloride (pH 8)–10 mM EDTA–5× Denhardt solution–30% (vol/vol) deionized formamide–0.1% (wt/vol) sodium dodecyl sulfate–0.1 mg of hydrolyzed yeast RNA per ml for 16 h at 42°C. The slot blots were washed in 0.09 M NaCl–9 mM Tris hydrochloride (pH 8)–1 mM EDTA–0.1% (wt/vol) sodium dodecyl sulfate at 50 to 55°C for oligodeoxynucleotide probes and at 82°C for genomic probes. Labeled probes which hybridized to the target nucleic acids on slot blots were visualized by autoradiography with Kodak X-OMAT film and Du Pont intensifying screens. One to seven days was required for visualization.

## RESULTS

**Design of species-specific oligodeoxynucleotide probes.** Sequencing efforts focused on bacteria which have been implicated in periodontal disease or are closely related to the potential pathogens. The bacteria used for sequencing or specificity determinations are listed in Table 1. Four rRNA regions were chosen for partial sequencing. Two of the sequencing primers used were as described by Lane et al. (12), and two other primers were novel and are described in Materials and Methods. By alignment of sequences for each region, hypervariable sequences were identified and species-specific oligodeoxynucleotide probes were designed. The oligodeoxynucleotide probe sequences are listed in Table 2.

**Analysis of species-specific oligodeoxynucleotides.** To determine whether or not the synthetic probes were specific for the appropriate microbe, each probe was tested against a panel of total nucleic acids isolated from 20 microorganisms common to the oral cavity (Table 1). Also tested were organisms not usually found in the oral cavity, such as *Escherichia coli* and *W. succinogenes*. In addition, the *W. recta* probes were tested against the *Campylobacter* species listed in Materials and Methods, since DNA homologies have been reported for *Wolinella* and *Campylobacter* species (19).

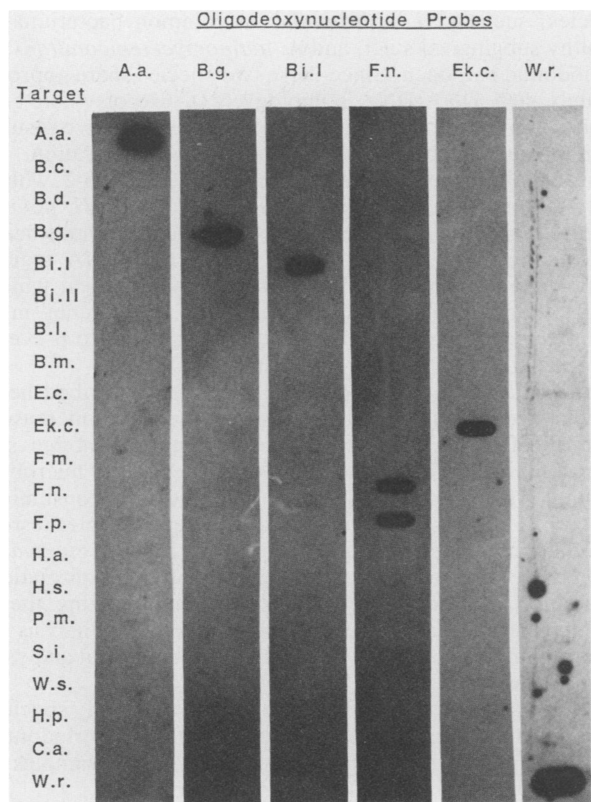


FIG. 1. Specificity panel for rRNA oligodeoxynucleotide probes. Oligodeoxynucleotide probes for six periodontal bacteria, *A. actinomycetemcomitans*, *B. gingivalis*, *B. intermedius* type I, *F. nucleatum*, *E. corrodens*, and *W. recta*, were isotopically labeled as described in Materials and Methods and hybridized to total nucleic acids from a group of potentially cross-hybridizing species. (The species names for the panel of bacteria are abbreviated as defined in Table 1.) The probes listed in Table 1 for each species were combined and tested as a species mixture.

The analysis indicated that all probes were species specific and did not cross-hybridize with closely related species, with one exception. The *F. nucleatum* probes were not specific. Probe Fn-1 cross-hybridized with *F. mortiferum* and *F. periodonticum* and Fn-2 and Fn-3 also hybridized to *F. periodonticum*. A summary of the specificities of the probes is presented in Table 2.

**Relative specificities of genomic and oligodeoxynucleotide probes.** A comparison of the relative specificities of the oligodeoxynucleotide probes and genomic probes is shown in Fig. 1 and 2. It is clear that the oligodeoxynucleotide probes can be designed to distinguish between closely related species which contain homologous DNA sequences, for example, *H. aphrophilus* and *A. actinomycetemcomitans* of *B. intermedius* types I and II (Fig. 1). In contrast, genomic probes from these microbes cross-hybridized to a significant degree (Fig. 2).

**Detection of periodontal bacteria in clinical samples.** Because periodontal bacteria can be pathogenic when present as 1% of the total bacteria inhabiting a plaque sample (16), direct detection of bacteria from plaque samples requires detection sensitivity as well as specificity. To determine the detection limit for oligodeoxynucleotide probes, purified nucleic acids from known quantities of *F. nucleatum* bacteria were hybridized against a mixture of labeled Fn-1, Fn-2,

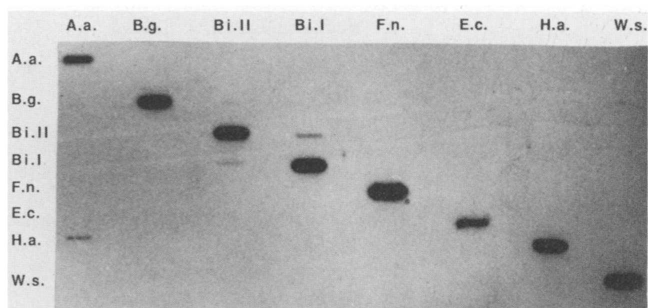


FIG. 2. Specificity panel for genomic DNA probes. Eight bacterial species, *A. actinomycetemcomitans*, *B. gingivalis*, *B. intermedius* types I and II, *F. nucleatum*, *E. corrodens*, *H. aphrophilus*, and *W. succinogenes*, were tested for cross-hybridizing sequences (species abbreviations are listed in Table 1). Total nucleic acids from each species were purified, and samples were immobilized onto Nytran. Genomic DNA was isotopically labeled as described in Materials and Methods. Each genomic probe was then hybridized to itself and to genomic DNAs from the seven other species.

and Fn-3 probes. Figure 3 shows the titration of signal intensity over a range of 0 to $4 \times 10^6$ *F. nucleatum* bacteria. The level of detection under these conditions was approximately 200 bacteria. With the probes listed in Table 2, the level of detection was approximately $10^3$ bacteria for the remaining bacteria listed in Table 2.

Since this level of detection was appropriate for measurement of bacteria from plaque samples, 17 plaque samples were tested with probes for *B. gingivalis*, *B. intermedius*, or *F. nucleatum*. These samples were from individuals with evidence of moderate or severe periodontal disease, as defined by pocket depths of 4 to 12 mm. Most of the samples hybridized to *F. nucleatum* probe Fn-1 (Fig. 4). This was not surprising, since *Fusobacterium* species are common oral bacteria (24). In contrast, only a subset of samples hybridized with the *B. gingivalis* or *B. intermedius* probes. Note

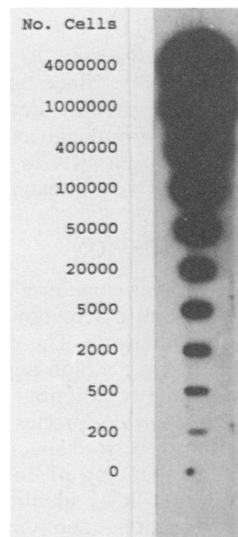


FIG. 3. Detection limits for isotopically labeled oligodeoxynucleotide probes. Total nucleic acids from microscopically counted *F. nucleatum* bacteria were diluted into human placental DNA, and the dilutions were immobilized onto Nytran membranes in a range of 0 to $4 \times 10^6$ bacteria per slot. The slotted material was hybridized to *F. nucleatum* oligodeoxynucleotide probes Fn-1, Fn-2, and Fn-3.

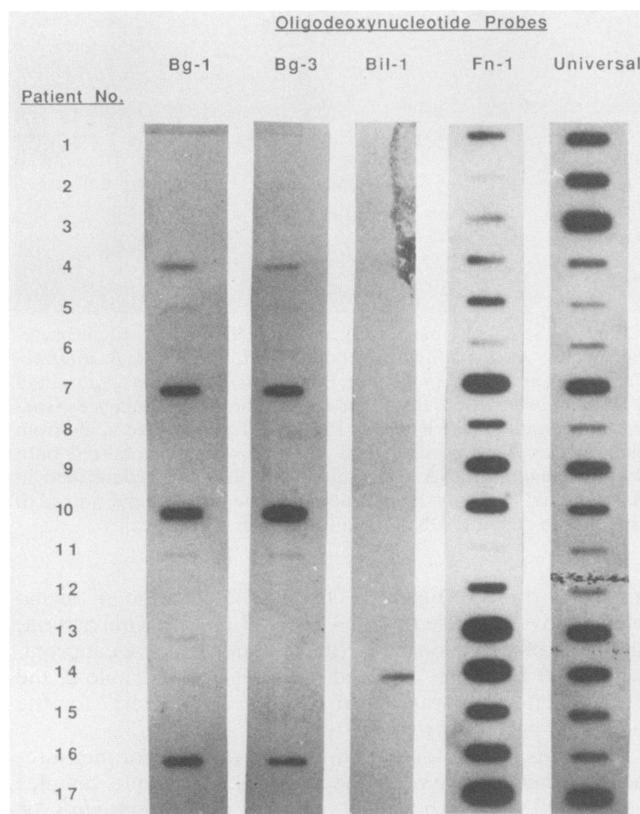


FIG. 4. Detection of bacteria from subgingival plaque samples of patients with periodontal disease. Total nucleic acids from 17 plaque samples were prepared and immobilized onto Nytran membranes. Each sample was then probed with $^{32}$P-labeled oligodeoxynucleotide probes for *B. gingivalis* (Bg-1 and Bg-3), *B. intermedius* type I (Bi-1), and *F. nucleatum* (Fn-1) and three universal sequencing probes (12). Autoradiographs were visualized at 6 h and 3 days for the universal-sequence-probed membranes and the remaining membranes, respectively.

that both Bg-1 and Bg-3 hybridized to the same plaque samples with the same intensity. Since both probes appear specific, *B. gingivalis* was probably detected directly in these samples from patients with diagnosed disease. Universal probes which hybridize to all rRNAs were a control to show the relative levels of rRNAs in each plaque sample.

## DISCUSSION

Elucidating the role of specific bacteria in the periodontal disease process requires accurate detection of specific microorganisms from mixed populations. The oligodeoxynucleotide probes described here have a high degree of specificity when hybridized with a panel of potential cross-hybridizing species. Only the *F. nucleatum* probes had unexpected cross-hybridizations with two related species, *F. mortiferum* and *F. periodonticum*. Sequencing of 16S rRNA from *F. periodonticum* revealed that it is identical in the region complementary to the three oligodeoxynucleotide probes. Sequencing of 16S rRNA from *F. mortiferum* revealed that the region complementary to Fn-1 contains one mismatch at the 3′ end of the probe.

These chemically synthesized probes were more specific than probes made from complete genomic sequences. This feature is critical in distinguishing between closely related species, such as *H. aphrophilus*, a common bacterium in healthy subgingival sulci, and *A. actinomycetemcomitans*, a periodontal pathogen. Since these two species share approximately 40% DNA-DNA homology (23), use of whole-cell probes can result in a high frequency of false-positive results with plaque samples or any other mixed population. In contrast, oligodeoxynucleotide probes Aa-2 and Ha-2, which were targeted to *A. actinomycetemcomitans* and *H. aphrophilus*, respectively, were species specific. The specificity derives from the sequence diversity in the rRNA region complementary to Aa-2 and Ha-2. The two probes, which target the same rRNA region, have six mismatches in a 24-base region (Table 2). This is easily sufficient to prevent hybridization to an inappropriate target (1).

An additional advantage to the approach described here may be the high detection sensitivities due to the increased number of rRNA targets relative to unique genomic sequences. High target levels may allow detection of microbes directly from plaque samples without the time-consuming process of culture. The small group of plaque samples tested demonstrated detectable levels of rRNAs from *B. gingivalis* and *B. intermedius* type I. Although no culture confirmation was done with these patients, other studies using these probes suggest that oligodeoxynucleotide probe analysis of plaque samples correlates well with microbiological analysis of the same samples (14).

The data demonstrate that these probes are highly specific and are suitable for use as tools for detection of periodontal microbes, whether in cultured isolates or plaque samples.

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## LITERATURE CITED

1. **Anderson, L. M., and B. D. Young.** 1985. Quantitative filter hybridisation, p. 73–111. *In* B. D. Hames and S. J. Higgins (ed.), Nucleic acid hybridisation. IRL Press, Washington, D.C.
2. **Chuba, P. J., K. Pelz, G. Krekeler, T. S. De Isele, and U. Gobel.** 1988. Synthetic oligodeoxynucleotide probes for the rapid detection of bacteria associated with human periodontitis. J. Gen. Microbiol. **134:**1981–1983.
3. **De Long, E. F., G. S. Wickham, and N. R. Pace.** 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. Science **243:**1360–1363.
4. **French, C. K., E. D. Savitt, S. L. Simon, S. M. Eklund, M. C. Chen, L. C. Klotz, and K. K. Vaccaro.** 1986. DNA probe detection of periodontal pathogens. Oral Microbiol. Immunol. **1:**58–62.
5. **Giovannoni, S. J., E. F. Delong, G. J. Olsen, and N. R. Pace.** 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. J. Bacteriol. **170:** 720–726.
6. **Gobel, U. B., A. Geiser, and E. J. Stanbridge.** 1987. Oligonucleotide probes complementary to variable regions of ribosomal RNA discriminate between *Mycoplasma* species. J. Gen. Microbiol. **133:**1969–1974.
7. **Holt, S. C., J. Ebersole, J. Felton, M. Brunsvold, and K. S. Kornonan.** 1988. Implantation of *Bacteroides gingivalis* in nonhuman primates initiates progression of periodontitis. Science **239:**55–57.
8. **Huysman, E., and R. De Wachter.** 1986. Compilation of small

ribosomal subunit RNA sequences. Nucleic Acids Res. **14:** 73–118.

9. **Johnson, J. L., and B. Harich.** 1986. Ribosomal ribonucleic acid homology among species of the genus *Bacteroides*. Int. J. Syst. Bacteriol. **36:**71–79.
10. **Kuritza, A. P., C. E. Getty, P. Shaughnessy, R. Hesse, and A. A. Salyers.** 1986. DNA probes for identification of clinically important *Bacteroides* species. J. Clin. Microbiol. **23:**343–349.
11. **Kuritza, A. P., and A. A. Salyers.** 1985. Use of a species-specific DNA hybridization probe for enumerating *Bacteroides vulgatus* in human feces. Appl. Environ. Microbiol. **50:**958–964.
12. **Lane, D. J., B. Pace, G. J. Olsen, D. A. Stahl, M. L. Sogin, and N. R. Pace.** 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. Proc. Natl. Acad. Sci. USA **82:**6955–6959.
13. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.).** 1985. Manual of clinical microbiology, 4th ed., p. 1056. American Society for Microbiology, Washington, D.C.
14. **Maiden, M., A. Tanner, S. McArdle, K. Najpauer, and J. M. Goodson.** 1989. Multi-center evaluation of tetracycline fiber therapy. IV. Dent. Res. **68:**241.
15. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual, p. 122–123. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Moore, W. E. C.** 1987. Review: microbiology of periodontal disease. J. Periodontal Res. **22:**335–341.
17. **Pace, N. R., G. J. Olsen, and C. R. Woese.** 1986. Ribosomal RNA phylogeny and the primary lines of evolutionary descent. Cell **45:**325–326.
18. **Roberts, M. C., S. L. Hillier, F. D. Schoenknecht, and K. K. Holmes.** 1985. Comparison of Gram stain, DNA probes, and culture for the identification of species of *Mobiluncus* in female genital specimens. J. Infect. Dis. **152:**74–77.
19. **Romaniuk, P. J., B. Zoltowska, T. J. Trust, D. J. Lane, G. J. Olsen, N. R. Pace, and D. A. Stahl.** 1987. *Campylobacter pylori*, the spiral bacterium associated with human gastritis, is not a true *Campylobacter* sp. J. Bacteriol. **169:**2137–2141.
20. **Sanger, G., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
21. **Savitt, E. D., M. N. Strzempko, K. K. Vaccaro, W. J. Peros, and C. K. French.** 1988. Comparison of cultural methods and DNA probe analyses for the detection of Actinobacillus actinomycetemcomitans, Bacteroides gingivalis, and Bacteroides intermedius in subgingival plaque samples. J. Periodontol. **59:**431–438.
22. **Schmidhuber, S., W. Ludwig, and K. H. Schleifer.** 1988. Construction of a DNA probe for the specific identification of *Streptococcus oralis*. J. Clin. Microbiol. **26:**1042–1044.
23. **Strzempko, M. N., S. L. Simon, C. K. French, J. A. Lippke, F. F. Raia, E. D. Savitt, and K. K. Vaccaro.** 1987. A cross-reactivity study of whole genomic DNA probes for *Haemophilus actinomycetemcomitans*, *Bacteroides intermedius*, and *Bacteroides gingivalis*. J. Dent. Res. **66:**1543–1546.
24. **Tanner, A., S. McArdle, and J. M. Goodson.** 1989. Multi-center evaluation of tetracycline fiber therapy. V. J. Dent. Res. **68:**197.
25. **Woese, C. R.** 1987. Bacterial evolution. Microbiol. Rev. **51:** 221–271.