Synthetic Oligodeoxynucleotide Probes for the Rapid Detection of Bacteria Associated with Human Periodontitis

By PAUL J. CHUBA,¹ KLAUS PELZ,¹ GISBERT KREKELER,²
TERESA S. DE ISELE¹ AND ULF GÖBEL^{1*}

¹ Institut für Medizinische Mikrobiologie und Hygiene der Universität Freiburg,
Hermann Herder Strasse 11, D-7800 Freiburg, FRG

² Zentrum für ZMK, Sektion Parodontalchirurgie, Hugstetter Strasse 55, D-7800 Freiburg, FRG

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Analysis of the subgingival microflora has recently implicated *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans* and several black *Bacteroides* species in the aetiology of juvenile, adult and rapidly progressing periodontitis. Rapid bacteriological diagnosis has been hampered by the slow growth and fastidious nature of these bacteria. To construct diagnostic probes, dideoxy sequencing of the 16S rRNA molecules from *A. (H.) actinomycetemcomitans*, *Haemophilus aphrophilus*, *Bacteroides gingivalis*, *Bacteroides intermedius* subgroup II, *Bacteroides asaccharolyticus* and several closely related species was performed. Next, oligodeoxynucleotides, complementary to defined regions of the 16S rRNA exhibiting considerable evolutionary divergence, were synthesized for use as molecular probes. In a dot-blot hybridization assay, all strains from each of the species for which probes were constructed were correctly identified, with a detection limit of less than 5×10^3 organisms. No cross-hybridization to closely related species (except for *H. aphrophilus* and *Haemophilus paraphrophilus*) or contaminating bacteria was observed. Using a modified DNA/RNA hybridization technique, the detection could be performed in less than 12 h, as compared to 2–3 weeks using conventional bacteriological procedures.

INTRODUCTION

Periodontal infections are a major cause of alveolar bone destruction and tooth loss worldwide. The aetiology and pathogenesis of periodontal infections in both juveniles and adults have recently come under intensive investigation (Haapasalo *et al.*, 1986; Moore *et al.*, 1984, 1987; Moore, 1987). Although up to 325 different bacterial species have been isolated from periodontal pockets (Moore, 1987), relatively few Gram-negative anaerobes and facultative anaerobes appear to be implicated in the process of subgingival colonization and pocket formation (Newman & Socransky, 1977; Slots, 1979; Slots *et al.*, 1985; Gmür *et al.*, 1986); however, more work needs to be done to establish statistical comparisons and to reconcile results from various laboratories (Moore *et al.*, 1987). Most closely associated with juvenile-onset periodontitis have been *Actinobacillus* (*Haemophilus*) *actinomycetemcomitans* (Cristersson *et al.*, 1985; Zambon, 1985), and *Capnocytophaga* species (Slots & Genco, 1984). Rapidly progressing periodontitis has been characterized by the presence of one or more of the organisms *Bacteroides gingivalis*, *A. (H.) actinomycetemcomitans*, *Fusobacterium nucleatum* and oral spirochaetes. Black-pigmented *Bacteroides* species, mainly *Bacteroides intermedius*, seem to be associated with the adult forms of periodontal disease (Duerden *et al.*, 1987). *Eikenella corrodens*, *Haemophilus aphrophilus* and *Actinomyces* species, among numerous others, have also been isolated from mixed infections in periodontitis (Moore *et al.*, 1985).

Although timely microbiological detection would be useful for the assessment of prognosis, for epidemiological studies, and for possible antimicrobial therapy in periodontal disease, rapid diagnosis has been hampered by the time required for conventional identification of the bacteria

involved. Several methods for the rapid detection of selected periodontal pathogens have been reported. These include immunofluorescence using monoclonal or polyclonal antibodies (Bonta *et al.*, 1985; Miyoshi *et al.*, 1986; Cristersson *et al.*, 1987*a b*), and DNA/DNA hybridization using either random cloned DNA fragments (Salysers *et al.*, 1983; Kuritza *et al.*, 1986) or whole genomic DNA isolated from the organisms of interest (French *et al.*, 1986; Strzempko *et al.*, 1987; Roberts *et al.*, 1987) as probes. DNA/RNA hybridization procedures for detection of pathogenic bacteria have also been developed (Falkow, 1985; Eisenstein & Engelberg, 1986; Göbel *et al.*, 1987*a, b*; Haun & Göbel, 1987; Viscidi & Yolken, 1987), and we describe here the application of a DNA/RNA dot-hybridization procedure for the species-specific detection of *A. (H.) actinomycetemcomitans*, *H. aphrophilus*, *B. gingivalis*, *B. intermedius* subgroup II and *B. asaccharolyticus*.

METHODS

Bacterial strains and media. Bacterial strains were obtained from the National Collection of Type Cultures Collection (NCTC), London, UK, from the Deutsche Stammsammlung für Mikroorganismen (DSM), Braunschweig, FRG, from our own collection and from the collection of Professor W. Mannheim (Marburg, FRG). The strain designations and original sources are given in Table 1. Although the suggestion has been made to move the species *Actinobacillus actinomycetemcomitans* into the genus *Haemophilus* (Potts *et al.*, 1985), the International Committee on systematic bacteriology has not yet favoured this proposal (Frederiksen, 1987), and we therefore refer to it here as *Actinobacillus (Haemophilus) actinomycetemcomitans*. *Bacteroides* species were identified using established bacteriological techniques. SDS-PAGE was used to differentiate *B. intermedius* I and *B. intermedius* II. YCB-blood agar [10% (v/v) defibrinated sheep blood, 1% (w/v) peptone, 0.5% (w/v) NaCl, 0.2% (w/v) meat extract, 0.5% (w/v) yeast extract, 0.03% (w/v) L-cysteine chloride monohydrate, 2% (w/v) glucose, 1.8% (w/v) Difco agar] supplemented with 0.001% (w/v) haemin and 0.0001% (w/v) vitamin K was used for anaerobic cultivation, Columbia green blood agar (Oxoid) was used for aerobic cultures, and Malachite green blood agar [5% (w/v) sheep blood, 3.8% (w/v) trypticase soya agar, 128 mg bacitracin l⁻¹, and 8 mg malachite green ml⁻¹] was used as a selective medium for *A. (H.) actinomycetemcomitans* and *H. aphrophilus* (Lennette *et al.*, 1985). Gas chromatography was routinely performed on anaerobic isolates.

Isolation of rRNA. Bulk cellular RNA was isolated as follows. Bacterial cultures (500 ml) were grown to mid-exponential phase in Brain Heart Infusion medium at 37 °C without shaking. *Actinobacillus* species were incubated in 5% (v/v) carbon dioxide, and *Bacteroides* species were grown using the Gas-Pak system (Merck). Cells were harvested by centrifuging at 6500 r.p.m. for 10 min at 4 °C in a Sorvall GSA rotor. The pellets were resuspended in 100 ml phosphate-buffered saline, and recentrifuged at 6500 r.p.m. for 10 min. The washed cells were resuspended in 20 ml 4 M-LiCl, 8 M-urea and incubated for 16 h at 0 °C (Auffray & Rougeon, 1980). After centrifugation at 10000 r.p.m. for 20 min in a Sorvall SS34 rotor, 10 ml TE buffer (TE is 0.01 M-Tris/HCl, 0.001 M-EDTA, pH 8.0) was added to the wet pellet. Next, two extractions with an equal volume of TE-saturated phenol, followed by a single extraction with 24:1 (v/v) chloroform/isoamyl alcohol, were performed. Finally, two volumes of ice-cold ethanol were added, and the RNA preparations stored at -20 °C.

Reverse transcriptase sequencing of 16S rRNAs. 16S rRNA sequence data were obtained using the dideoxy chain termination method (Sanger *et al.*, 1977). Bulk cellular RNA served as template, and synthetic deoxyoligonucleotides complementary to conserved regions of the 16S rRNAs were used as 'universal' primers (see below), together with avian myeloblastoma virus reverse transcriptase (Pharmacia), essentially as described by Lane *et al.* (1985) and Pace *et al.* (1986). The final concentrations of deoxy and dideoxy nucleotides in the reaction mixes were as follows: dNTPs, 100 µM; ddCTP, 12 µM; ddTTP, 12 µM; ddATP, 5 µM; ddGTP, 8 µM.

Synthesis of deoxyoligonucleotides. Deoxyoligonucleotides, for use as sequencing primers or hybridization probes (15–32 bases in length), were synthesized on a solid support with an Applied Biosystems 380A DNA synthesizer using diisopropylphosphoramidite chemistry. Oligonucleotides were purified by electrophoresis on native 20% (w/v) acrylamide gels, followed by electroelution as described previously (Göbel *et al.*, 1987*c*). The oligonucleotide primers (with which the sequence data discussed here were obtained) had the sequences 5' CCCGTC AATTCCTTTGAGTTT 3' and 5' TACCAGGGTATCTAATCCTGTT 3', and were complementary to the 'U4' and 'U5' conserved regions of the prokaryotic 16S rRNA molecule, respectively (Gray *et al.*, 1984). The sequences of oligonucleotides used for probe construction are given in Fig. 1.

Hybridizations. For 'slot-blot' hybridization of bacterial cultures, whole cells were resuspended in 1 ml 0.15 M-NaCl to a final concentration of approximately 5×10^7 cells ml⁻¹, and samples of 50 µl, 25 µl and 10 µl were blotted directly (without further handling) onto nylon membranes (Pall) using the Minifold I and Minifold II filtration manifolds (Schleicher and Schuell). Filters were subsequently baked for 2 h at 80 °C. Oligonucleotide probes were end-labelled with [γ -³²P]ATP (5000 Ci mmol⁻¹, 185 TBq mmol⁻¹; Amersham) using T4 polynucleotide kinase (BRL) according to the recommendations of the supplier, and purified on 20% polyacrylamide gels followed by electroelution. Filters were prehybridized for 30 min at 65 °C in a hybridization

Probe BG-32: comparison of *B. gingivalis* and *B. fragilis*
V8 variable region

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3'ATCCTCAAACGCTATATGGCAGTTCTGAAGGTG 5'          DNA BG-32
5'AUGAUUACUAGGAGUUUGCGAUUACCGUCAAGCUCCACAGCGAAAGCGUUA 3'RNA B.g.
5'AUGAAUACUCGUGUUUGCGAUUACAGUA-AGCGGCCA-AGCGAAAGCAUUA 3'RNA B.f.
      *  * *              *  * *  *  *

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Probe BI-31: comparison of *B. intermedius* and *B. fragilis*
V7 variable region

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3'GGACTCATGTGCGTCGCTCCGCCTTAAGTA 5'          DNA BI-31
5'CUGUCAGACCUGAGUACACGCAGCGCAGGCGGAAUUCAGUGUGUAGCGGUGA 3'RNA B.i.
5'CUGUCAGUCUUGAGUACAGUAGAGUGGGCGGAAUUCGUGUGUAGCGGUGA 3'RNA B.f.
      *  *          * * * * *  *

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Probe BA-30: comparison of *B. asaccharolyticus* and *B. fragilis*
V7 variable region

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3'GAGTACGATCTCACATGACCTCCATGCACC 5'          DNA BA-30
5'AAACUCUCUCAUGCUAGAGUGUACUGGAGGUACGUGGNNCGUGUGUG 3'RNA B.a.
5'UGAUACUGUCAGUCUUGAGUACAGUAGAGUGGGCGGAAUUCGUGUG 3'RNA B.f.
      * * *  *  * *  *  * *  *  * *  *  *

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Probes AAC-25 and HA-25: comparison of *A. (H.) actinomycetemcomitans*, '*A. hominis*'
and *H. aphrophilus*

V8 variable region

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3'CCCTTAACCCCAAATCGGGACCACG 5'          DNA AAC-25
3'AACACGAAACTCGAACACGGACATCGA 5'          DNA HA-28
5'GUCGAUUUGGGGAUUGGGGUUAGCCUGGUGCCCNAAAGCUAACGUGAU 3'RNA A.ac.
5'GUGCAUUUGGGGAUUGGCUUUGAGCUUGGUGCCUGUAGCUAACGUGAU 3'RNA H.a.
5'GUCGAUUUGGGGAUUGGACUGUGAGUCUGGUGCCCUAAGCUAACGUGAU 3'RNA A.h.
      * * *  *  * * *  * * *

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Fig. 1. Comparison of representative partial 16S rRNA sequences corresponding to variable regions V7 and V8. The *Bacteroides fragilis* (Weisburg *et al.*, 1985) sequence was compared with the *Bacteroides* sequences determined here to select the complementary oligonucleotide probes BG-32, BI-31, and BA-30. *Actinobacillus hominis*, *A. (H.) actinomycetemcomitans*, and *H. aphrophilus* sequences were compared to select the probes AAC-25 and HA-28. Positions of nonidentity are indicated with an asterisk; N = ambiguous. B.g., *B. gingivalis*; B.f., *B. fragilis*; B.i., *B. intermedius* II; B.a., *B. asaccharolyticus*; A.ac., *A. (H.) actinomycetemcomitans*; A.h., '*A. hominis*'; H.a., *H. aphrophilus*.

buffer consisting of $5 \times \text{SSC}$ ($1 \times \text{SSC}$ is 0.15 M-NaCl, 0.015 M-sodium citrate), $5 \times$ Denhardt's solution ($1 \times$ Denhardt's solution is 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone and 0.02% Ficoll), 0.2% SDS, and 250 mg yeast tRNA ml⁻¹. Subsequently, $1-2 \times 10^6$ c.p.m. of end-labelled oligonucleotide in hybridization buffer was applied to each filter, and hybridization was allowed to proceed for 1-2 h at 65°C. After hybridization, each filter was washed once for 2 min at 65°C in $5 \times \text{SSC}$, 0.2% SDS, air dried, and autoradiography was performed using Kodak X-OMAT film with intensifier screens (2-12 h) at -70°C.

In order to determine the sensitivity of the probes in detecting bacteria on filters, serial dilutions were tested for colony-forming units (c.f.u.), and in parallel, samples were applied to filters and hybridized as described above. To test the influence of the presence of contaminating bacteria on the species-specific hybridization, exponential-phase liquid cultures (5×10^7 cells ml⁻¹) of *Bacteroides intermedius* II and *Bacteroides gingivalis* were diluted serially, mixed in various ratios as shown in Fig. 2, applied to filters, and hybridized using species-specific probes to one or the other organism.

RESULTS

16S rRNA sequencing

Bulk cellular rRNA isolated from *A. (H.) actinomycetemcomitans* NCTC 9710, *H. aphrophilus* NCTC 5906, '*A. hominis*' NCTC 11529, *B. gingivalis* DSM 20709, *B. intermedius* II NCTC 9336 and *B. asaccharolyticus* NCTC 9337 were used as templates in chain-termination sequencing reactions using oligonucleotide primers and reverse transcriptase. Partial 16S rRNA sequences obtained were compared with those of closely related organisms, including *B. fragilis* for the black *Bacteroides* species (Weisburg *et al.*, 1985), for the selection of species-specific probes. The relevant rRNA sequence alignments are shown in Fig. 1. For the purposes of this analysis, the

Table 1. *Bacterial strains and probe hybridizations*

Species	Collection no.*	Source and/or date	Hybridization with deoxyoligonucleotides				
			AAC-25	HA-28	BG-32	BI-31	BI-30
<i>Actinobacillus</i> (<i>Haemophilus</i>) <i>actinomycetemcomitans</i>	NCTC 9710 [†]	Lung abscess, Copenhagen, 1955	+	—	—	—	—
	Y4	Human	+	—	—	—	—
	B658(82)	Endocarditis, Würzburg, 1982	+	—	—	—	—
	Krüpe 6	Brain abscess, Fulda, 1985	+	—	—	—	—
	ATCC 29522	Mandibular abscess	+	—	—	—	—
	Krüpe 912	Heart prosthesis, Fulda, 1985	+	—	—	—	—
	AA944/86	Oral isolate, Würzburg, 1986	+	—	—	—	—
	B1463/86	Blood culture, Würzburg, 1987	+	—	—	—	—
	CCUG 8810A	Oral isolate, Sweden, 1983	+	—	—	—	—
	CCUG 19546	Sweden, 1987	+	—	—	—	—
<i>Haemophilus aphrophilus</i>	NCTC 5906 [†]	Endocarditis, London, 1929	—	+	—	—	—
	NCTC 5886	Endocarditis, London, 1939	—	+	—	—	—
	Stuttgart 390	Brain abscess, Stuttgart, 1984	—	+	—	—	—
	CCUG 11832A	Endocarditis, Sweden, 1981	—	+	—	—	—
	252035 B	Oral isolate, Marburg, 1983	—	+	—	—	—
	WS12533/85	Abscess, Frankfurt, 1985	—	+	—	—	—
<i>Haemophilus paraphrophilus</i>	NCTC 10556	Abscess, Leeds, 1968	—	+	—	—	—
	05333/82	Sputum, Bonn, 1982	—	+	—	—	—
<i>Bacteroides intermedius</i> II	NCTC 9336	Gingivitis, London, 1954	—	—	—	+	—
	NCTC 9338	Empyema, London, 1954	—	—	—	+	—
	NG4600	Labial abscess, Freiburg, 1987	—	—	—	+	—
	NG50	Cervix, Freiburg, 1987	—	—	—	+	—
	E4228	Abscess, Freiburg, 1987	—	—	—	+	—
	Sp2516	Lung, Freiburg, 1987	—	—	—	+	—
	NG492	Vaginal endometritis, Freiburg, 1987	—	—	—	+	—
	E13808	Osteomyelitis (jaw), Freiburg, 1987	—	—	—	+	—
	E10460	Wound, Freiburg, 1987	—	—	—	+	—
	E1439	Submucosal abscess, Freiburg, 1987	—	—	—	+	—
	LindI	Periodontitis, Freiburg, 1987	—	—	—	+	—
	LindII	Periodontitis, Freiburg, 1987	—	—	—	+	—
	E14193	Submucosal abscess, Freiburg, 1987	—	—	—	+	—
	E5491	Submucosal abscess, Freiburg, 1987	—	—	—	+	—
	E10961	Submucosal abscess, Freiburg, 1987	—	—	—	+	—

Table 1. (continued)

Species	Collection no.*	Source and or date	Hybridization with deoxyligonucleotides				
			AAC-25	HA-28	BG-32	BI-31	BI-30
<i>Bacteroides asaccharolyticus</i>	NCTC 9337	Haemorrhoid, London, 1954	-	-	-	-	+
	NG4611	Placenta, Freiburg, 1986	-	-	-	-	+
	NG1331	Vaginitis, Freiburg, 1987	-	-	-	-	+
	E4698	Wound, Freiburg, 1987	-	-	-	-	+
	E15413	Abscess, Freiburg, 1987	-	-	-	-	+
	NG256	Endometritis (cervix), Freiburg, 1987	-	-	-	-	+
	NG504	Vaginitis, Freiburg, 1987	-	-	-	-	+
	NG1479	Cervix carcinoma, Freiburg, 1987	-	-	-	-	+
	DSM 20709 ^a	Subgingival isolate, 1980	-	-	+	-	-
<i>Bacteroides gingivalis</i>	12EA1	Periodontitis, Freiburg, 1987	-	-	+	-	-
	16EA1	Periodontitis, Freiburg, 1987	-	-	+	-	-
	15EA1	Periodontitis, Freiburg, 1987	-	-	+	-	-
	E1270/I	Periodontitis, Freiburg, 1987	-	-	+	-	-
	E1270/II	Periodontitis, Freiburg, 1987	-	-	+	-	-

The following strains were also tested, and did not react with any of the five probes: *Actinobacillus equuli* NCTC 8529^a, *Actinobacillus haemolyticus* subsp. *indologenes* NCTC 11313, *Actinobacillus hominis* NCTC 11529^a, *Actinobacillus lignieresii* NCTC 4976, *Haemophilus influenzae* NCTC 8143, *Eikenella corrodens* NCTC 10596^a, *Pasteurella multocida* NCTC 10322^a, *Bacteroides vulgatus* DSM 1447^a, *Bacteroides ovatus* DSM 1896^a, *Bacteroides distans* DSM 20516^a, *Bacteroides denticola* DSM 20614^a, *Bacteroides melaninogenicus* NCTC 11321, *Bacteroides bivia* DSM 20514, *Bacteroides buccalis* DSM 20616, and *Bacteroides intermedius* I NG726, NG302 and NG303.

* Strains marked NCTC were from the National Collection of Type Cultures, London, UK. Strains labelled DSM were from the Deutsche Stammsammlung für Mikroorganismen, Braunschweig, FRG. Strains isolated in Freiburg were from our own strain collection. All other strains were from W. Mannheim (Marburg, FRG). Type strains are denoted by a superscript t.

Fig. 2. Effect of contaminating bacteria on the oligonucleotide hybridization assay (probes BG-32 and BI-31). Hybridizations of fivefold dilutions of suspensions of *B. gingivalis* and *B. intermedium* II mixed and applied to filters in various ratios are shown.

variable region V7 and V8 sequences (*E. coli* 16S rRNA nomenclature according to Gray *et al.*, 1984) were considered. The sequences selected for the construction of oligonucleotide probes AAC-25 (for *A. (H.) actinomycetemcomitans*), HA-28 (for *H. aphrophilus*), BG-32 (for *B. gingivalis*), BI-31 (for *B. intermedium*), and BA-30 (for *B. asaccharolyticus*) are also given in Fig. 1.

Probe specificity

The results of slot-blot hybridization experiments using the oligonucleotide probes described above are shown in Table 1. A number of species belonging to the genera *Bacteroides*, *Haemophilus* and *Actinobacillus*, as well as several more distantly related bacteria, were tested. Oligonucleotide BG-32 hybridized only to *B. gingivalis* (Table 1). The *B. gingivalis* type strain was identified as were five clinical isolates. Oligonucleotide AAC-25 identified the *A. (H.) actinomycetemcomitans* type strain as well as nine strains from the collection of W. Mannheim (Marburg, FRG). Oligonucleotide HA-28 hybridized with both *H. aphrophilus* (six strains) and *H. paraphrophilus* (two strains), indicating a close genotypic similarity between the two species. Phenotypically, these species differ only in the requirement for V factor. Oligonucleotide BI-31 hybridized specifically with *B. intermedium* subgroup II (15 strains), but did not recognize three strains of *B. intermedium* subgroup I. These subgroups may represent different species (Johnson & Harich, 1966). Oligonucleotide BG-30 hybridized with *B. asaccharolyticus* NCTC 9337, and with seven clinical isolates. None of the probes reacted with any of the other species listed in Table 1. All of the clinical strains isolated in Freiburg (Table 1) which tested positive for one of the probes were also verified by phenotypic testing.

Probe sensitivity

To determine the number of bacteria which could be detected using these probes, viable count determinations were performed on exponential-phase cultures, dilutions of which were then applied to hybridization filters. Each of the five probes detected between 1×10^3 and 5×10^3 c.f.u. (data not shown). This is in good agreement with the detection limit of about 1×10^3 c.f.u. for probes specific for the genus *Proteus* previously determined in this laboratory (Haun & Göbel, 1987).

Although the probes showed no cross-reactivity when bacteria from pure cultures were tested, it was possible that the presence of closely related bacteria in mixed or contaminated cultures could affect the specificity or sensitivity of the hybridization. Fig. 2 shows that neither the sensitivity nor the specificity of hybridization of *B. gingivalis* with oligonucleotide BG-32 at various concentrations was affected by the presence of *B. intermedium* II at various concentrations, nor was hybridization of *B. intermedium* II with oligonucleotide BI-31 affected by the presence of *B. gingivalis*.

DISCUSSION

Definitive microbiological analysis of multiple samples from periodontitis patients has hitherto been very time consuming and expensive. Although tests based on immunofluorescence are in development, and DNA/DNA hybridization tests have been described (see Introduction), identification of the obligate and facultative anaerobes associated with periodontitis is currently based on biochemical tests. This usually requires a period of two weeks between obtaining pure cultures and reporting results, primarily due to the fastidious nature of the bacteria involved and the complexity of the subgingival flora. Using the hybridization probes described here, the detection of bacteria in multiple samples could be performed rapidly (12 h), using a simple assay. The oligonucleotide probes AAC-25, BG-32, BI-31 and BA-30 were species-specific for *A. (H.) actinomycetemcomitans*, *B. gingivalis*, *B. intermedius* II and *B. asaccharolyticus* respectively, and oligonucleotide HA-28 hybridized with two closely related species *H. aphrophilus* and *H. paraphrophilus* (Table 1).

The approach described here combines important attributes of rapid molecular sequencing and hybridization methods for diagnostic applications. (1) The unique architecture of the 16S rRNA allows direct sequencing. Molecular cloning is therefore unnecessary, as is the screening of large numbers of DNA fragments for specificity in hybridization reactions. (2) Automated synthesis is now widely available to construct defined oligonucleotide probes, in contrast to whole genomic DNA, where the quality may vary from one preparation to another. (3) The target molecule for the probes is the 16S rRNA molecule, present in very high copy numbers (10^4 molecules per cell, compared with only one copy for most prokaryotic genes), allowing for favourable hybridization kinetics and high sensitivity (detection limit about 5×10^3 bacteria). (4) Unlike whole genomic DNA probes directed against the same species, which tend to cross-react with closely related species (Strzempko *et al.*, 1987), four out of five of the oligonucleotides described here showed no cross-reactivity, and one probe (HA-28) cross-reacted only with a single very close relative (Table 1). (5) Short hybridization times (1–2 h for oligonucleotides versus 12–24 h for large DNA fragments of whole genomic DNA probes), exact prediction of hybridization conditions (Conner *et al.*, 1983), and easy handling of large sample numbers, make oligonucleotide hybridization assays attractive for both rapid diagnosis and extended epidemiological studies of periodontal infections.

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