Use of Synthetic Oligonucleotide DNA Probes for Identification and Direct Detection of *Bacteroides forsythus* in Plaque Samples

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Oligonucleotide DNA probes complementary to the hypervariable region of the 16S rRNA of *Bacteroides forsythus* were tested for their specificity and sensitivity against reference and clinical isolates of 73 different species of bacteria. The probes were used to detect the organism directly from nucleic acid extracts of subgingival plaque samples, and the results were compared with results of detection by culture methods. The data demonstrate that the probes are very specific (100%) and sensitive (100% when they are compared with those obtained by the culture method) and could reliably detect the organism in plaque samples. When a probe to a conserved region of the 16S rRNA (UP9A) was used to estimate the quantity of bacteria present in plaque samples, it gave results comparable to those of culture methods. The data suggest that total microbial load may be a parameter in periodontal disease.

Recently, Tanner et al. (22) described a new species of *Bacteroides*, *B. forsythus*, which was associated with periodontal disease. *B. forsythus* is an obligately anaerobic fusiform organism which is difficult to culture and identify. Recent studies suggest that *B. forsythus* is most closely related to *Bacteroides distasonis*; however, additional studies are required before the taxonomic status of this organism can be elucidated (4).

Since our understanding of the etiology of periodontal disease is limited by the necessity to use cumbersome and expensive culture techniques, various DNA probes have been developed for use in detecting suspected periodontal pathogens. Whole-cell DNA probes are valuable reagents for the identification of pure cultures of *Bacteroides* spp. (13, 19). However, these probes were not useful for the detection of specific organisms in samples, such as subgingival plaque, where there are high numbers of closely related species (9, 11). More recently, short oligonucleotide probes complementary to the hypervariable regions of 16S rRNA were shown to be sensitive and specific enough to detect particular species of bacteria in samples containing a large variety and numbers of other organisms (3, 11).

The preparation and characterization of a panel of oligonucleotide probes for the detection of six periodontal pathogens have been reported previously (3, 11). In order to prove the usefulness of these probes, it is necessary to demonstrate sensitivity and specificity on pure cultures of microorganisms and to compare the detection of a given bacterium by a DNA probe with detection by conventional microbial culture techniques. In this report, we describe the use of probes for *B. forsythus* to detect the organism in subgingival plaque samples. These probes are highly specific and gives results comparable to those of conventional methods.

MATERIALS AND METHODS

Subjects. Subjects for these studies were considered disease-free if they did not have any subgingival pockets of greater than 5 mm in depth or sites which exhibited bleeding on probing. Diseased sites had pocket depths of >5 mm and

exhibited bleeding on probing. There were five subjects in each group, and four samples were taken from each subject.

Organisms. Reference strains were obtained from the American Type Culture Collection (ATCC) and were verified biochemically (see Table 1). *Campylobacter* species were kindly provided by F. C. Tenover, Veterans Administration Medical Center, Seattle, Wash.

Oral clinical isolates of *Bacteroides*, *Porphyromonas*, *Prevotella*, *Wolinella*, and *Fusobacterium* were obtained at the University of Washington Graduate Periodontics Clinic from patients with various forms of periodontal disease. Subgingival plaque samples were taken from 5-mm- to 10-mm-deep periodontal pockets with a Morse scaler, placed in 2 ml of prerduced Hanks balanced salt solution containing 10% (vol/vol) heat-inactivated horse serum, and transported to an anaerobic glove box within 10 min. The plaque samples were dispersed by a 5-s ultrasonic pulse from a microultrasonic cell disrupter (Kontes). Samples were serially diluted in the same medium and plated onto prerduced blood agar plates for bacterial identification and enumeration of the total anaerobic CFU. The plates were incubated for 7 to 10 days in an anaerobic environment at 35°C, and organisms were identified to the species level as described previously (5–7).

Pure clinical isolates and reference strains were grown on blood agar plates for 2 to 3 days or until good growth occurred and were then removed from plates with a sterile cotton-tipped swab and dispersed into sterile saline. Cells were sedimented and lysed with 200 µl of MicroProbe Lysing Solution (MicroProbe Corp., Bothell, Wash.) and stored at –70°C until they were needed.

For comparison of DNA probe and culture techniques, samples were taken as described above and placed into 1 ml of prerduced salts solution (containing 0.038 M sodium chloride, 1.073 mM potassium chloride, 2.05 mM sodium thiosulfate, reazurin sodium salt [0.0125 mg/liter], and 4.125 mM L-cysteine-free base), dispersed by sonication, and then plated and incubated as described above. After samples were removed for culture, a 10% (vol/vol) volume of 100 mM EDTA and 100 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was added and thor-

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TABLE 1. Species which did not react with *B. forsythus* probes

Organism	Strain	Organism	Strain
Bacteroides spp.		<i>Bacteroides gracilis</i> (18)	ATCC 33236
<i>B. fragilis</i>	ATCC 23745	<i>Bacteroides ureolyticus</i>	ATCC 33387
<i>B. caccae</i>	ATCC 43185		
<i>B. distasonis</i>	ATCC 8503	Other microorganisms	
<i>B. eggerthii</i>	ATCC 27754	<i>Capnocytophaga gingivalis</i>	ATCC 33624
<i>B. merdae</i>	ATCC 43184	<i>Capnocytophaga ochracea</i> (20) ^c	ATCC 33596
<i>B. ovatus</i>	ATCC 8483	<i>Capnocytophaga sputigena</i>	ATCC 33612
<i>B. stercoris</i>	ATCC 43183	<i>Fusobacterium gonidiaformans</i>	ATCC 25563
<i>B. thetaiotaomicron</i>	ATCC 29741	<i>Fusobacterium nucleatum</i> (30)	ATCC 25586
<i>B. uniformis</i>	ATCC 8492	<i>Fusobacterium periodonticum</i>	ATCC 33693
<i>B. vulgatus</i>	ATCC 8482	<i>Eubacterium saburreum</i>	ATCC 33271
		<i>Actinobacillus actinomycetem-</i>	
Porphyromonas spp.		<i>comitans</i>	ATCC 29523
<i>P. gingivalis</i> (16) ^a	ATCC 33277	<i>Haemophilus aphrophilus</i>	ATCC 33389
<i>P. asaccharolytica</i>	ATCC 25260	<i>Haemophilus paraphrophilus</i>	ATCC 29241
<i>P. endodontalis</i> (6)	ATCC 35406	<i>Haemophilus segnis</i>	ATCC 33393
		<i>Eikenella corrodens</i>	ATCC 23834
Prevotella		<i>Wolinella succinogenes</i>	ATCC 29543
<i>P. melaninogenica</i>	ATCC 25845	<i>Wolinella curva</i>	ATCC 35224
<i>P. bivia</i>	ATCC 29303	<i>Wolinella recta</i> (25)	ATCC 33238
<i>P. buccae</i>	ATCC 33574	<i>Escherichia coli</i>	BJM lab strain
<i>P. buccalis</i>	ATCC 35310	<i>Peptostreptococcus micros</i>	ATCC 33270
<i>P. corporis</i> (18)	ATCC 33547	<i>Selenomonas noxia</i>	ATCC 43541
<i>P. denticola</i>	ATCC 33185	<i>Selenomonas flueggeii</i>	ATCC 43531
<i>P. disiens</i>	ATCC 29426	<i>Streptococcus intermedius</i>	ATCC 27335
<i>P. heparinolytica</i>	ATCC 35895	<i>Streptococcus sputigena</i>	ATCC 35185
<i>P. intermedia</i>	ATCC 33563	<i>Streptococcus sanguis</i>	ATCC 10556
<i>P. intermedia</i> (22)	ATCC 25611	<i>Streptococcus mutans</i>	ATCC 25175
<i>P. loescheii</i>	ATCC 15930	<i>Actinomyces viscosus</i>	ATCC 19246
<i>P. oralis</i>	ATCC 33269	<i>Actinomyces israelii</i>	ATCC 12102
<i>P. oris</i>	ATCC 33573	<i>Actinomyces israelii</i>	ATCC 23860
<i>P. oulora</i>	ATCC 43324		
<i>P. veroralis</i>	ATCC 33779	Campylobacter species^d	
<i>P. zoogeleoformans</i>	ATCC 33285	<i>C. coli</i>	PS1068 (ATCC 33559)
<i>Prevotella species^b</i> (9)		<i>C. cryaerophilia</i>	PS4581 (ATCC 43158)
Unidentified species ^c (12)		<i>C. hyointestinalis</i>	PS4506 (ATCC 35217)
		<i>C. mucosalis</i>	PS4519 (NCTC 11000)
Organisms of uncertain		<i>C. nitrofigilis</i>	PS4520 (ATCC 33309)
generic position		<i>C. sputorum</i> subsp. <i>bubulus</i>	PS4518 (ATCC 33562)
<i>Bacteroides putredinis</i>	ATCC 29800	<i>C. pylori</i>	(NCTC 11637)
<i>Bacteroides splanchnicus</i>	ATCC 29572	<i>C. upsaliensis</i>	PS4511 (NCTC 11541)
<i>Bacteroides capillosus</i>	ATCC 29799	<i>C. jejuni</i>	PS1025
<i>Bacteroides levii</i>	ATCC 29147	<i>C. laridis</i>	PS4510 (NCTC 11352)
<i>Bacteroides macacae</i>	ATCC 33141	<i>C. concisus</i>	ATCC 33237
<i>Bacteroides fucosus</i>	ATCC 25662		

^a The number of clinical isolates of the indicated species tested is given in parentheses.

^b *Prevotella* species were isolates which were *P. melaninogenica*, *P. loescheii*, or *P. denticola*.

^c Species of anaerobic black-pigmented rods which could not be identified biochemically.

^d Original sources of strains and acquisition numbers are given in parentheses. NCTC, National Collection of Type Cultures (Central Public Health Laboratory, London, England).

oughly mixed. Samples were placed immediately on dry ice and were then stored at -70°C until they were needed.

Preparation of samples and DNA probes. Oligonucleotide probes complementary to hypervariable regions of the 16S rRNA from *B. forsythus* and a universal priming sequence probe (UP9A) were provided by MicroProbe Corp. A combination of two such probes for *B. forsythus* was used in these studies: Bf2, which has been described previously (3), and Bf8, the sequence of which is proprietary. Nucleic acids were extracted from the plaque samples by using phenol-chloroform-isoamyl alcohol as described previously (11). The nucleic acids were applied to Nytran filters (Schleicher & Schuell, Keene, N.H.), washed with 200 μl of $6\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and allowed to hybridize with the probes as described previously

(3, 11). Pure bacterial samples in 200 μl of MicroProbe Lysing Solution were diluted with 60 μl of $20\times$ SSC (3.0 M NaCl plus 0.30 M sodium citrate [pH 7.0]) and 52 μl of formalin, mixed with a vortex mixer, and then heated at 55°C for 15 min. After heating, 20 μl of the sample was diluted with 580 μl of $15\times$ SSC containing 25% formalin and mixed thoroughly, and 103 μl was applied to the filters. Filter strips were allowed to hybridize with the probes as described previously (11). To control for the spurious loss of target nucleic acids and false-positive results, portions of each sample were analyzed with the use of a universal priming probe sequence as described previously (11). To estimate the total microbial load or the number of *B. forsythus* in each sample by DNA probe analysis, nucleic acids were extracted and then the total nucleic acids were measured by compar-

TABLE 2. Detection of *B. forsythus* by the DNA probe and culture methods in subgingival plaque samples for samples in which the two methods did not agree^a

Sample no.	DNA probe (log ₁₀ <i>B. forsythus</i> /sample) ^b	Anaerobic CFU (log ₁₀) ^c
2	5.301	8.477 (0.07)
4	5.699	7.954 (0.55)
6	5.699	8.699 (0.10)
7	6.477	7.845 (4.00)
9	6.301	7.301 (10.00)

^a *B. forsythus* was not detected in any of the samples by culture methods.

^b Estimated by DNA probe analysis, as described in the text.

^c Values in parentheses are the percentage of total anaerobic *B. forsythus* CFU. Values for *B. forsythus* were taken from the estimate by DNA probe analysis.

ison with a nucleic acid standard after autoradiography, as described previously (11). For these experiments, *Prevotella intermedia* and *B. forsythus* were grown in liquid cultures to the midlogarithmic stage of growth, washed three times in saline, and suspended to a density of 10⁹ cells per ml (direct microscopic count with a Petroff-Hausser chamber). Nucleic acids were extracted from these suspensions and were used as standards.

RESULTS

The *B. forsythus* probes were tested against the ATCC reference strains and clinical isolates listed in Table 1. No hybridization was observed with any of 74 different species except *B. forsythus*. The probes hybridized with 40 *B. forsythus* isolates from humans and 6 *B. forsythus*-like isolates obtained from monkeys (*Macaca fascicularis*) (1). The overall sensitivity of these probes was 100% and the specificity was 100% when they were compared with those of biochemical identification. Plaque samples were also analyzed by DNA probe and conventional culture methods. DNA probes detected *B. forsythus* in 18 of 20 samples from diseased sites, while the organism was detected in only 13 samples by culture. In cases in which the organism was not detected by culture but was detected by DNA probe, the organism was found to be a minor component of the flora at the site (Table 2). In samples 7 and 9 (Table 2), the organism was present as a major component of the anaerobic flora. *B. forsythus* was not detected by either method in 20 samples taken from patients without periodontal disease (data not shown).

When it was used to estimate the microbial load, the universal primer probe yielded results comparable to those obtained by plate counts. In all individual cases, the values obtained by each method were in agreement within 1 log unit. The number of CFU (plate count) from disease sites ranged from 5.0 × 10⁶ to 5.0 × 10⁸ CFU per sample ($\bar{x}$ = 5.2 × 10⁷). The estimates by DNA probe analysis ranged from 2.0 × 10⁶ to 2 × 10⁸ cells per sample ($\bar{x}$ = 3.1 × 10⁷). The values obtained from samples from healthy sites ranged from 8.0 × 10⁴ to 1.0 × 10⁸ CFU per sample (plate count method, $\bar{x}$ = 2.9 × 10⁶) and 1.0 × 10⁵ to 2.0 × 10⁷ cells per sample (DNA probe method, $\bar{x}$ = 1.8 × 10⁶).

DISCUSSION

Despite intense investigation in past years, the etiologic parameters of periodontal diseases are poorly understood. Numerous putative pathogens have been implicated, but to

date, none has emerged as the key pathogen. Until recently, culture techniques were the only available methods for such studies. Polyclonal and monoclonal antibodies are useful for identification of these organisms, but they may not detect all members of a given species (2, 23).

More recently, the concept of using bacterial enzyme activities, such as trypsinlike enzyme or sialidase, and host-derived activities (aspartate aminotransferase) as indicators of disease has gained recognition (9, 12, 17). However, because these enzymes are also present in many bacteria, they are not useful for the detection of a specific etiologic agent.

DNA hybridization can detect pathogens in primary culture or directly from clinical specimens, such as feces, pus, or vaginal washes (18). It has been shown (3, 11) that the whole-cell DNA probes do not possess the specificity required to detect oral pathogen directly from highly mixed samples such as subgingival plaque samples but that they are reliable for use on pure cultures.

The data presented here demonstrate that oligonucleotide DNA probes complementary to the hypervariable region of the 16S rRNA provide an alternative to whole-cell DNA probes, specific antibodies, or conventional methods for the study of pure cultures or plaque samples. The *B. forsythus* probes were observed to correctly identify all pure isolates of *B. forsythus* and did not cross-react with any of the 74 other species tested.

In previous studies (11, 13, 19), probe specificities have been examined by testing cross-reactions with closely related species. Since the taxonomy of *B. forsythus* was unknown when these studies were initiated, we tested the *B. forsythus* probes against *Campylobacter* species as well as against organisms commonly encountered in plaque samples. Recent studies (4) suggest that *B. forsythus* is most closely related to *B. distasonis*, a member of the *B. fragilis* group. Therefore, the probes were tested against these organisms as well. As demonstrated in Table 1, no cross-reactions were observed. These data confirm and extend our previous studies (3) in which probe Bf2 did not hybridize with nucleic acids from *Prevotella corporis*, *Prevotella denticola*, *Prevotella loescheii*, *Escherichia coli*, *Fusobacterium mortiferum*, *Fusobacterium periodonticum*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus*, *Haemophilus segnis*, *Peptostreptococcus micros*, *Streptococcus intermedius*, *Wolinella succinogenes*, or *Candida albicans* (3). These data therefore demonstrate that these probes are specific for *B. forsythus*.

Having demonstrated that the probes were specific for this organism, the reliability in detecting *B. forsythus* directly from plaque samples was investigated. In the 19 healthy sites, *B. forsythus* was not detected by DNA probe or culture. This supports earlier observations suggesting that *B. forsythus* is associated with periodontal disease but not with a healthy state (23). In sites from patients with periodontal disease, *B. forsythus* was detected in 13 samples by the DNA probe and culture methods and in 5 samples by the probe method but not by the culture method, but it was absent by both methods in 2 samples. The results for samples in which positive results were obtained by the DNA probe method, but not by the culture method, illustrate both the advantages of DNA probes and the difficulty in proving their sensitivity and specificity. It is possible that these samples represent false-positive results with the DNA probes; however, we know of no other bacteria which hybridize with these probes. Therefore, these results most likely demonstrate the increased sensitivity of the probes compared with that of

culture. The flora in these types of samples is usually very complex; more than 360 species have been isolated from such sites (15). We usually characterize between 25 and 35 colony types per site. From our work and that of others (16), we know that it is difficult to detect by the culture method organisms which represent 1% or less of the flora. The five samples in which *B. forsythus* was detected by the probe method but not by the culture method exemplify the problem. The estimates of CFU of *B. forsythus* by probe analysis all represented less than 1% of the total CFU except for samples 7 and 9 (4 and 10%, respectively). These data suggest that the discrepancy between the two methods is the result of the insensitive nature of culture methods and is not a false-positive result with the DNA probes.

The universal probe was found to give excellent estimations of the total microbial load when compared with that given by anaerobic plate counts. In all cases, the values obtained by probe and culture for each sample were within 1 log unit of each other. For these comparisons, only the anaerobic plate counts were used, but the aerobic plate counts in all cases were <10% of the values obtained for the anaerobic counts. Given the inherent error in samplings and other indeterminate errors, the values obtained by each method are in excellent agreement. The averages of all samples by each method also are in good agreement.

If the growth rates of the organisms used for the preparation of the standards (logarithmic) were substantially different from those of the organisms in the plaque samples, a close agreement in the estimate of microbial number would not be expected since the number of rRNA copies would be substantially different. However, the close agreement between the estimates made by the DNA probe and plate count methods suggests that subgingival plaque bacteria are in a state of rapid growth.

Over the past 5 years we have performed anaerobic plate counts on samples from 80 patients with periodontal disease; the average count in all samples was 2.3×10^7 CFU per site. This is close to the average value in the present study (5.2×10^7 CFU per site). This apparent difference may reflect the difference in recovery of the two transport media used, Hanks balanced salts solution with horse serum and pre-reduced salts solution (this study). Therefore, the data obtained in this study by DNA probe analysis are consistent with those obtained in previous work.

Most anaerobic infections, including periodontal diseases, have a mixed flora. Recent studies have suggested that periodontal diseases are caused by different combinations of organisms (21). It has long been known that there is a shift in the proportion of microorganisms in the flora as a site progresses from a healthy to a diseased state (15). Moore et al. (14) have reported that the composition of the flora shifts through a progression of different species as the health status progresses from health to gingivitis to periodontal disease, and there are specific organisms which appear to be unique for each condition. We propose here that there is another parameter involved in these diseases, that of microbial load.

We observed that the microbial load for diseased sites was 18-fold greater than that for healthy sites (on the basis of DNA probe estimations). This is not altogether unexpected, since diseased sites have deeper pocket depths with presumably larger volumes; therefore, it is reasonable to assume there would be more material to collect during sampling. It should be noted that two samples from healthy sites had anaerobic plates counts of $\sim 10^8$ CFU. Although culture confirmations for other periodontal pathogens were not performed, the samples were tested with DNA probes to

Actinobacillus actinomycetemcomitans, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Wolinella recta*. One sample had low levels of *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia* II (ATCC 33563 homology group), and *Eikenella corrodens*; but the other sample had no detectable pathogens (data not shown). These data suggest either that these sites were misdiagnosed or that there is another parameter involved. Misdiagnosis is most likely, since clinical parameters are not reliable for the diagnosis of disease (8). The other parameter may be the requirement for a specific concentration of pathogens in the site prior to disease initiation.

The model is consistent with the vast majority of data relating to the etiology of periodontal disease. Numerous groups have reported an association of different putative periodontal pathogens (15). Most of these putative pathogens are not very pathogenic in other systems; for example, *Porphyromonas gingivalis* has a very high 50% infective dose in mice (10). The lipopolysaccharide of this organism is also less toxic than other endotoxins are (20). Other pathogens cause clinical infections at other body sites in immunocompromised humans, but rarely in healthy individuals. Thus, many of these organisms have a low pathogenic potential. Our model postulates that periodontal disease activity results when the overall pathogenic potential of a given site reaches a threshold level. This threshold is reached at different levels of specific organisms at a given site. We therefore propose that periodontal diseases may be a progression of microbial species in various combinations and that disease is proposed to be the result of a combination of microbial cohorts and the microbial load at a given site.

In summary, we observed that oligonucleotide DNA probes for *B. forsythus* are sensitive and specific reagents for the identification of pure isolates of the organism and detection in plaque samples. The DNA probes could be used to give a reliable estimate of the number of bacteria present in a sample.

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REFERENCES

1. Braham, P. H., and B. J. Moncla. Submitted for publication.
2. de Macario, E. D., and A. I. Macario. 1985. Monoclonal antibodies for bacterial identification and taxonomy: 1985 and beyond. Clin. Lab. Med. 5:531-540.
3. Dix, K., S. M. Watanabe, S. McArdle, D. I. Lee, C. Randolph, B. Moncla, and D. E. Schwartz. 1990. Species-specific oligonucleotide probes for the identification of periodontal bacteria. J. Clin. Microbiol. 28:319-323.
4. Fraser, G. J., F. E. Dewhirst, and B. J. Paster. 1990. Abstr. 68th Ann. Meet. Int. Assoc. Dent. Res. abstr. 2156, p. 378. 1990.
5. Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.). 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
6. Holdeman, L. V., E. P. Cato, and W. E. C. Moore. 1984. Taxonomy of anaerobes: present state-of-the-art. Rev. Infect. Dis. 6:S3-S10.
7. Holdeman, L. V., R. W. Kelly, and W. E. C. Moore. 1984. Family I. *Bacteroidaceae* Pribram 1933, 10^{AL}, p. 602-662. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
8. Kornman, K. S. 1986. Nature of periodontal diseases: assess-

- ment and diagnosis. *J. Periodontal Res.* **22**:192–204.
9. Loesche, W. J. 1986. The identification of bacteria associated with periodontal disease and dental caries by enzymatic methods. *Oral Microbiol. Immunol.* **1**:65–70.
 10. Mayrand, D., and S. C. Holt. 1988. Biology of black-pigmented *Bacteroides* species. *Microbiol. Rev.* **52**:134–152.
 11. Moncla, B. J., P. Braham, K. Dix, S. Watanabe, and D. Schwartz. 1990. Use of synthetic oligonucleotide DNA probes for the identification of *Bacteroides gingivalis*. *J. Clin. Microbiol.* **28**:324–327.
 12. Moncla, B. J., P. Braham, and S. L. Hillier. 1990. Sialidase (neuraminidase) activity among gram-negative anaerobic and capnophilic bacteria. *J. Clin. Microbiol.* **28**:422–425.
 13. Moncla, B. J., L. Strockbine, P. Braham, J. Karlinsey, and M. C. Roberts. 1988. The use of whole-cell DNA probes for the identification of *Bacteroides intermedius* isolates in a dot blot assay. *J. Dent. Res.* **67**:1267–1270.
 14. Moore, L. V. H., W. E. C. Moore, E. P. Cato, R. M. Smibert, J. A. Burmeister, A. M. Best, and R. R. Ranney. 1987. Bacteriology of human gingivitis. *J. Dent. Res.* **66**:989–995.
 15. Moore, W. E. C. 1987. Review: microbiology of periodontal disease. *J. Periodontal Res.* **22**:335–341.
 16. Moore, W. E. C., L. V. Holdeman, R. M. Smibert, I. J. Good, J. A. Burmeister, K. G. Palcanis, and R. R. Ranney. 1982. Bacteriology of experimental gingivitis in young adult humans. *Infect. Immun.* **38**:651–667.
 17. Persson, G. R., T. A. DeRouen, and R. C. Page. 1990. Relationship between gingival crevicular fluid levels of aspartate aminotransferase and active tissue destruction in treated chronic periodontitis patients. *J. Periodont. Res.* **25**:81–87.
 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 *Mobiluncus* spp. in female genital specimens. *J. Infect. Dis.* **152**:74–77.
 19. Roberts, M. C., B. J. Moncla, and G. E. Kenny. 1987. Chromosomal DNA for the identification of *Bacteroides* species. *J. Gen. Microbiol.* **133**:1423–1430.
 20. Slots, J., and R. J. Genco. 1984. Microbial pathogenicity: black-pigmented *Bacteroides* species, *Capnocytophaga* species, and *Actinobacillus actinomycetemcomitans* in human periodontal disease: virulence factors in colonization, survival and tissue destruction. *J. Dent. Res.* **63**:412–421.
 21. Socransky, S. S., A. D. Haffagee, and J. L. Dzink. 1988. Relationship of subgingival microbial complexes to clinical features at the sampled sites. *J. Clin. Periodontol.* **15**:440–444.
 22. Tanner, A. C. R., M. A. Listgarten, J. L. Ebersole, and M. N. Strzempko. 1986. *Bacteroides forsythus* sp. nov., a slow-growing fusiform *Bacteroides* sp. from the human oral cavity. *Int. J. Syst. Bacteriol.* **36**:213–221.
 23. Tompkins, L. S. 1985. Recombinant DNA and other direct specimen identification techniques. *Clin. Lab. Med.* **5**:99–114.