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Whole genomic and randomly-cloned DNA probes for two fastidious periodontal pathogens, *Porphyromonas gingivalis* and *Bacteroides forsythus* were labeled with digoxigenin and detected by a colorimetric method. The specificity and sensitivity of the whole genomic and cloned probes were compared. The cloned probes were highly specific compared to the whole genomic probes. A significant degree of cross-reactivity with *Bacteroides* species. *Capnocytophaga* sp. and *Prevotella* sp. was observed with the whole genomic probes. The cloned probes were less sensitive than the whole genomic probes and required at least 10^6 target cells or a minimum of 10 ng of target DNA to be detected during hybridization. Although a ten-fold increase in sensitivity was obtained with the whole genomic probes, cross-hybridization to closely related species limits their reliability in identifying target bacteria in subgingival plaque samples.

Keywords

Porphyromonas gingivalis, Bacteroides forsythus, DNA probes, non-isotopic labeling

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Comparison of randomly cloned and whole genomic DNA probes for the detection of *Porphyromonas gingivalis* and *Bacteroides forsythus*

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Abstract

Whole genomic and randomly-cloned DNA probes for two fastidious periodontal pathogens, *Porphyromonas gingivalis* and *Bacteroides forsythus* were labeled with digoxigenin and detected by a colorimetric method. The specificity and sensitivity of the whole genomic and cloned probes were compared. The cloned probes were highly specific compared to the whole genomic probes. A significant degree of cross-reactivity with *Bacteroides* species. *Capnocytophaga* sp. and *Prevotella* sp. was observed with the whole genomic probes. The cloned probes were less sensitive than the whole genomic probes and required at least 10^6 target cells or a minimum of 10 ng of target DNA to be detected during hybridization. Although a ten-fold increase in sensitivity was obtained with the whole genomic probes, cross-hybridization to closely related species limits their reliability in identifying target bacteria in subgingival plaque samples.

Keywords

Porphyromonas gingivalis; *Bacteroides forsythus*; DNA probes; non-isotopic labeling

Porphyromonas gingivalis and *Bacteroides forsythus* are considered to be pathogens in adult periodontitis (1, 2). However, the detection of these bacteria by conventional culturing methods is usually time consuming and technically difficult due to the fastidious nature of these bacteria. The use of specific DNA sequences as probes can be a rapid means of detecting a particular bacterial species. Radiolabeled or non-isotopically labeled whole genomic probes have been used by a number of investigators to detect *P. gingivalis* and *B. forsythus* (3–9). One problem related to the use of whole genomic probes has been the cross-hybridization of genetically unrelated species which may share many chromosomal sequences with the target species. To be useful in identifying and quantifying a given bacterial species in a mixed population, it is essential that the DNA probe be species specific. Whole genomic DNA probes work well when pure cultures are analyzed under conditions where cell concentration and hybridization are carefully controlled. However, false-positive signals may result when whole genomic probes are used to analyze subgingival plaque samples directly (22). Another potential problem in using whole genomic probes is the presence of plasmid sequences that might be related to other bacterial species. If the plasmid is integrated into the chromosome, it could cause unwanted hybridization (10).

Cloning of specific chromosomal DNA fragments is another approach for making DNA probes (11–15). Randomly cloned probes are obtained by cleaving the chromosomal DNA

into smaller fragments with restriction endonucleases and inserting these fragments into a cloning vector. Randomly cloned probes have been used for the identification of *Bacteroides* sp. such as *B. thetaiotaomicron* 11) and *B. fragilis* 12) and have been developed for the identification of oral bacteria such as *Peptostreptococcus micros* 13) and the spirochetes *T. denticola, T. socranskii, T. vincentii* and *T. pectinovorum* 14). Randomly cloned fragments from *A. actinomycetemcomitans* have also been used for studying the epidemiology of this bacterium in localized juvenile periodontitis (15).

Although whole genomic probes have been used in a number of studies to detect *P. gingivalis* and *B. forsythus*, non-specific hybridization to other species requires the development of more specific probes for these two microorganisms. The objective of this study was to compare the specificity and sensitivity between cloned and whole genomic probes for the detection of *P. gingivalis* and *B. forsythus*.

Material and methods

The bacterial strains used in this study are listed in Table 1. *Porphyromonas* sp., *Prevotella* sp. and *Bacteroides* sp. were grown in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, MD) supplemented with hemin (5 μg/ml) and vitamin K (0.5 μg/ml) at 35°C in an anaerobic chamber (Coy Manufacturing, Ann Arbor, MI) containing an atmosphere of 80% N_2, 10% CO_2 and 10% H_2. A trypticase soy medium containing 1.5% agarose (Sigma Chemical, St. Louis, MO) supplemented with hemin (5 μg/ml) and 5% sheep blood was used for bacteria which grew poorly in broth. N-acetylmuramic acid (Sigma Chemical Co.) (10 μg/ml) was added to the trypticase soy blood agarose medium (BBL Microbiology Systems) for the growth of *B. forsythus*.

DNA was extracted according to a method modified from that of Lunsford and Macrina (16). Chromosomal DNA from *Prophyromonas gingivalis* ATCC 33277 and *B. forsythus* FDC 338 was used for making the whole genomic DNA probes. Chromosomal DNA was digested with *HindIII* (Boehringer-Mannheim Biochemicals, Indianapolis, IN; 2 units of enzyme/μg of DNA) at 37°C for 2 h and was ligated to pUC19, digested with the same enzyme, using T4 DNA ligase (Boehringer-Mannheim Biochemicals) according to the manufacturer's instructions. The ligated DNA was used to transform *E. coli* TB1 (Δ (*lac-pro*), *strA, ara, thi*, Ø80d*lacZ*ΔMl5, *hsdR*) which was made competent by treatment with $CaCl_2$ (17). The transformants were plated on LB medium [1% bacto-tryptone (Difco Laboratories, Detroit, MI), 0.5% bacto-yeast extract (Difco Laboratories), 1% NaCI] containing 50 μg/ml of ampicillin and 5-bromo-4 chIoro-3-indolyl β-D-galactoside (X-Gal; Fisher Scientific, Pittsburgh, PA) and incubated overnight. The white colonies were picked for further analysis. Plasmid DNA was extracted from the clones by the method of Holmes & Quigley (18) and was examined on 0.7% agarose gels following digestion with *Hin*dIII. Clones containing the largest *P. gingivalis* or *B. forsythus* insert DNA fragments were selected for the ability to hybridize to DNA from homologous strains but not to DNA from heterologous species. The randomly cloned DNA fragments and whole genomic DNA from *P. gingivalis* ATCC 33277 and *B. forsythus* FDC 338 were labeled with digoxigenin using the Genius™ system (Boehringer-Mannheim Biochemicals). The labeled probes were stored at −20°C until used.

Chromosomal DNA was denatured, for slot blot analysis, in 200 μl of 0.4 M NaOH-10 mM EDTA by boiling in a water bath for 10 min. The samples were then neutralized by adding an equal volume of 2 M ammonium acetate. Nylon membranes (Magnagraph, Micron Separation, Westboro, MA) were pre-wet with 6XSSC (1X SSC contains 0.15 M sodium chloride-15 mM sodium citrate, pH 7.0). The DNA samples were applied to the membrane using a Bio-slot blot apparatus (Bio-Rad Laboratories, Richmond, CA). One μg of DNA was

routinely used for slot blot analysis. Ten pg to 1 μg of DNA was applied to each well in the experiments used to determine the sensitivity of the DNA probes. The wells of the slot blot apparatus were then rinsed with 0.5 ml of 2X SSC and the membrane was air-dried and baked for 2 hours at 80°C.

In the experiments designed to determine the sensitivity of the DNA probes, pure cultures of *P. gingivalis* ATCC 33277 and *B. forsythus* FDC 338, ranging from 10^1 to 10^8 cells, were applied under vacuum to a nylon membrane in the slot blot apparatus. The cells were lysed *in situ* and the DNA denatured by placing the membrane on top of a pad of Whatman filter paper which had been saturated with 0.5 M NaOH-1.5 M NaCl. After 15 min the filter was placed on a second pad of filter paper saturated with 1.5 M NaCl-1.0 M Tris-HCl (pH 8.0). The filter was left in position for 15 min and was then incubated in a solution of proteinase K (Fisher Scientific; 100 μg/ml in distilled water) for 30 min at 37°C. The membrane was air-dried and baked at 80°C for 2 h (3). The blots were then hybridized with the labeled cloned probes or the whole genomic probes to determine the limits of detection.

Membranes were incubated at 68°C for 2 h in a pre-hybridization solution containing 5X SSC, 1% blocking agent, 0.1% N-laurylsarcosine, and 0.02% SDS. The pre-hybridization solution was replaced by a hybridization solution containing heat denatured probe DNA (10–20 ng/ml) and was incubated at 68°C overnight. The membranes were washed twice in 2X SSC-0.1% SDS for 5 min and twice in 0.1 X SSC-0.1% SDS for 15 min at 65°C. Following the post-hybridization washes, detection of the probe was performed as described in the manufacturer's instructions.

Results

Isolation and characterization of randomly cloned probes

Two of the hybrid plasmids, pPG98 and pBF169 hybridized specifically to 18 strains of *P. gingivalis* and 6 strains of *B. forsythus*, respectively, and showed no cross-species hybridization (Table 1). Based on their specificities, these two hybrid plasmids were further characterized and used throughout this study. Plasmid pPG97 was chosen from a library of 100 recombinant plasmids and contained a 2.2 kb *Hin*dIII fragment from *P. gingivalis* ATCC 33277 (Fig. 1, lane 2). Plasmid pBF169, from a collection of 176 clones, contained a 2.8 kb *Hin*dIII fragment from *B. forsythus* FDC 338 (Fig. 1, lane 3). The pUC19 vector sequence present in these hybrid plasmids did not hybridize to any of the 78 strains of oral bacteria used in this study.

Specificity of the cloned probes and whole genomic probes

To compare the specificity of the whole genomic probes and the cloned DNA fragments and pBF169), both types of probe were hybridized to DNA from the various oral species. A comparison of the *P. gingivalis* probes is shown in Figure 2. The whole genomic probe, obtained from strain ATCC 33277, hybridized to DNA from the 18 *P. gingivalis* strains (Fig. 2A, A1–C6). The strength of the hybridization reaction with the control strain DNA was observed in well Al. In contrast, weakly cross-hybridizations were evident with DNA from many of the other oral species including *A. actinomycetemcomitans, B. forsythus, Bacteroides capillosus, Bacteroides heparinolyticus, Bacteroides oulorum, Bacteroides zoogleoformans, Campylobacter curva, Campylobacter rectus, Capnocytophaga ochracea, Capnocytophaga gingivalis, Capnocytophaga sputigena, Prevotella buccae, Prevotella denticola, Prevotella nigrescens, Prevotella loescheii, Prevotella melaninogenica, Prevotella oralis, Porphyromonas asaccharolytica* and *Prevotella heparinolytica* (Fig. 2A, D1–H4). The pPG97 probe hybridized to DNA from all 13 *P. gingivalis* strains on the slot blot (Fig. 2B, E1–G1). The control DNA was present in well E1. An extremely faint reaction was

observed with DNA from *C. gingivalis* B2), *C. ochracea* B4) and *E. corrodens* 373 (C1). However, no hybridization was observed with DNA from the remaining 31 oral species applied to the blot. These results are summarized in Table 1.

The results of hybridizations with the whole genomic probes from *B. forsythus* FDC 338 and pBF169 are shown in Fig. 3. The whole genomic and cloned probes reacted strongly with 5 of 6 strains of *B. forsythus* Fig. 3A, B, respectively). The strength of the hybridization reactions with the control strain DNA was evident in wells D1 and A1, respectively. DNA from *B. forsythus* FDC 2008 hybridized weakly to both the whole genomic and cloned probes (Fig. 3A, D4; Fig. 3B, A4). However, the genomic probe hybridized to DNA from many of the oral species represented on the blot. A weak cross-hybridization could be detected with 11 of the *P. gingivalis* strains. In contrast, the pBF169 probe showed a barely detectable reaction with *C. gingivalis, E. corrodens* 373 and *P. gingivalis* M90-1279 (Fig. 3B, slots B2, C1 and G1, respectively).

Sensitivity of the cloned probes and whole genomic probes

The sensitivity of the cloned DNA probes for target bacteria in pure cultures was tested by using ten-fold serial dilutions of the *P. gingivalis* and *B. forsythus* cells on slot blots. Fig. 4 shows that the detection limits of the cloned DNA probe of *P. gingivalis* was 10^6 cells (Fig. 4, C2). Longer incubation times were required to enhance color development and lowered the detection limit to 10^5 cells (D2). The sensitivity of the cloned probes was also determined by hybridization of the probes to serially diluted total nucleic acid (10 pg to 1 μg extracted from homologous species. The digoxigenin-labeled pPG97 probe detected a minimum of 10 ng of nucleic acid (Fig. 4, D3). Increased sensitivity was obtained with the whole genomic probe compared to the cloned probe (Fig. 4, columns 1 and 4). Although the whole genomic probe could detect a ten-fold lower amount of DNA (Ing; Fig. 4, C4), a positive signal was also observed with the negative control which consisted of 1 μg DNA from *B. forsythus* Fig. 4, H4). To improve the sensitivity, the concentration of the labeled probe DNA was increased from 10 ng/ml to 100 ng/ml. However, the detection limit of the probe was not improved and high backgrounds, due to non-specific binding, were observed. The limits of detection of pBF169 were similar to those obtained with pPG97. The cloned probe detected 10^6 cells and 10 ng DNA and was one log less sensitive than the whole genomic probe.

Discussion

The present work shows that the cloned *P. gingivalis* and *B. forsythus* fragments can be used successfully, in slot blot assays, to distinguish each of these species from other bacteria associated with human periodontitis. The randomly cloned probes are more specific than probes made from complete genomic DNA. A significant degree of cross-reactivity was observed in this study with digoxigenin-labeled *P. gingivalis* and *B. forsythus* whole genomic probes. Similar results were obtained by others (4, 19). In those studies, a ^{32}P-labeled *P. gingivalis* whole-genomic DNA probe cross-hybridized with *P. asaccharolytica, P. denticola, P. endodontalis, B. forsythus, P. intermedia* and *P. oris*. In contrast to the present study, Tay et al. (7) showed no hybridization of a digoxigenin-labeled *P. gingivalis* whole genomic probe to other test species except for a weak reaction with one strain of *Neisseria*. In a separate study, a *B. forsythus* digoxigenin-labeled whole genomic probe did not hybridize to 75 strains representing 24 bacterial species (9). A bisulfite modified whole genomic *P. gingivalis* probe also showed Limited cross-reactions and only hybridized with *P. intermedia* and *H. aphrophilus* 8). In the three former studies DNA probes were tested against 10^5 to 10^6 bacterial cells of different species rather than against purified DNA.

Weak cross-reactivity between *P. gingivalis* and *B. forsythus* was shown in both the study of Savitt et al. (4) and in this study when whole genomic probes were used. The *B. forsythus* whole genomic probe hybridized weakly to 10 *P. gingivalis* strains, a finding that suggests a phylogenetic relationship between the two species. Analyzing the volatile and non-volatile fatty acids produced by *B. forsythus*, Braham and Moncla (20) reported that the metabolic end products of *B. forsythus* were virtually identical to those produced by *P. gingivalis*, although the proportions did not appear to be the same. They proposed that *B. forsythus* might be more closely related to the genius *Porphyromonas* than was previously suspected. Recently, Paster et al. (21) studied the phylogeny of *Bacteroides, Prevotella*. and *Porphyromonas* spp. by 16S rRNA sequence comparison analysis. The *Porphyromonas* cluster was divided into two subclusters, the first subcluster contained *P. gingivalis* and the second *B. forsythus*. The similarity between the two species was 87.5%. Because of the phylogenetic depth, the author questioned whether *B. forsythus* should not be considered a species of *Porphyromonas* instead of being assigned to a separate genus.

The detection limits of the *P. gingivalis* and *B. forsythus* randomly cloned probes were only 10^6 cells in the present study. Cloned probes, representing sequences other than ribosomal genes, may have limited sensitivity because bacterial cells usually contain only one copy of the target sequence. French et al. (22) reported a 10-fold decrease in sensitivity of cloned probes of *A. actinomycetemcomitans* in comparison with whole genomic probes. The limits of detection were $2X10^5$ for both randomly cloned *T. denticola* or *T. socranskii* probes after exposure of the blot for 3 days (14). A cloned probe for *B. thetaiotaomicron* could detect as little as 50 ng of bacterial DNA. At least 10^7 cells of *B. thetaiotaomicron* were required to produce a hybridization reaction which is detectable by autoradiography (11). Kuritza et al. (12) used three ^{32}P-labeled cloned DNA probes for the detection of *B. fragilis* directly in clinical specimens. The concentration of *Bacteroides* species had to be at least 10^6 cells in the mixed cultures.

Another consideration in using cloned probes is the degree of genetic diversity among strains within a bacterial species. The chromosomal region from which the probe generated has to be conserved in order to detect all strains of target bacteria. The existence of different clonal types of *P. gingivalis* have been shown (23). Bodinka et al. (24) used PCR to amplify the collagenase gene (*prtC*) from *P. gingivalis* ATCC 33277. Only 16 of 21 clinical isolates of *P. gingivalis* yielded a PCR product. The data indicated that not all *P. gingivalis* strains have the *prtC* gene and that nucleotide heterogeneity existed among *P. gingivalis* strains that had the *prtC* gene. *Bacteroides forsythus* also showed a considerable degree of genetic diversity. Using the arbitrarily primed polymerase chain reaction (AP-PCR), 24 *B. forsythus* genotypes were identified among 27 strains (9). In the present study, pPG97 and pBF169 hybridized to DNA from all homologous strains tested. The results suggested that the sequences of the cloned fragments are conserved. However, mismatches between the probe nucleic acids and the target sequence can occur due to the diversity of the gene sequence among strains. Base pair mismatching can affect the binding of the DNA probe (25).

Sensitivity of detection can be improved by PCR to increase the number of target sequences in the original samples (26). Since the randomly cloned probes for *P. gingivalis* and *B. forsythus* isolated and used in this study proved to be specific, sequencing the clones could provide valuable information for the synthesis of oligonucleotide primers which could be used for the PCR reaction.

In conclusion, the randomly cloned probes for *P. gingivalis* and *B. forsythus* were shown to be highly specific as compared to the whole genomic probes. It is important to emphasize this difference since a significant number of reports in the literature continue to describe the exclusive use of un-validated whole genomic probes for microbial identification. However,

it should also be emphasized that the cloned probes required 10^6 target cells for detection. Amplification of the target sequences by PCR may enhance the sensitivity. Since these two randomly selected clones have proved to be specific for the homologous bacteria, they may provide specific sequences for the synthesis of primers for PCR based applications.

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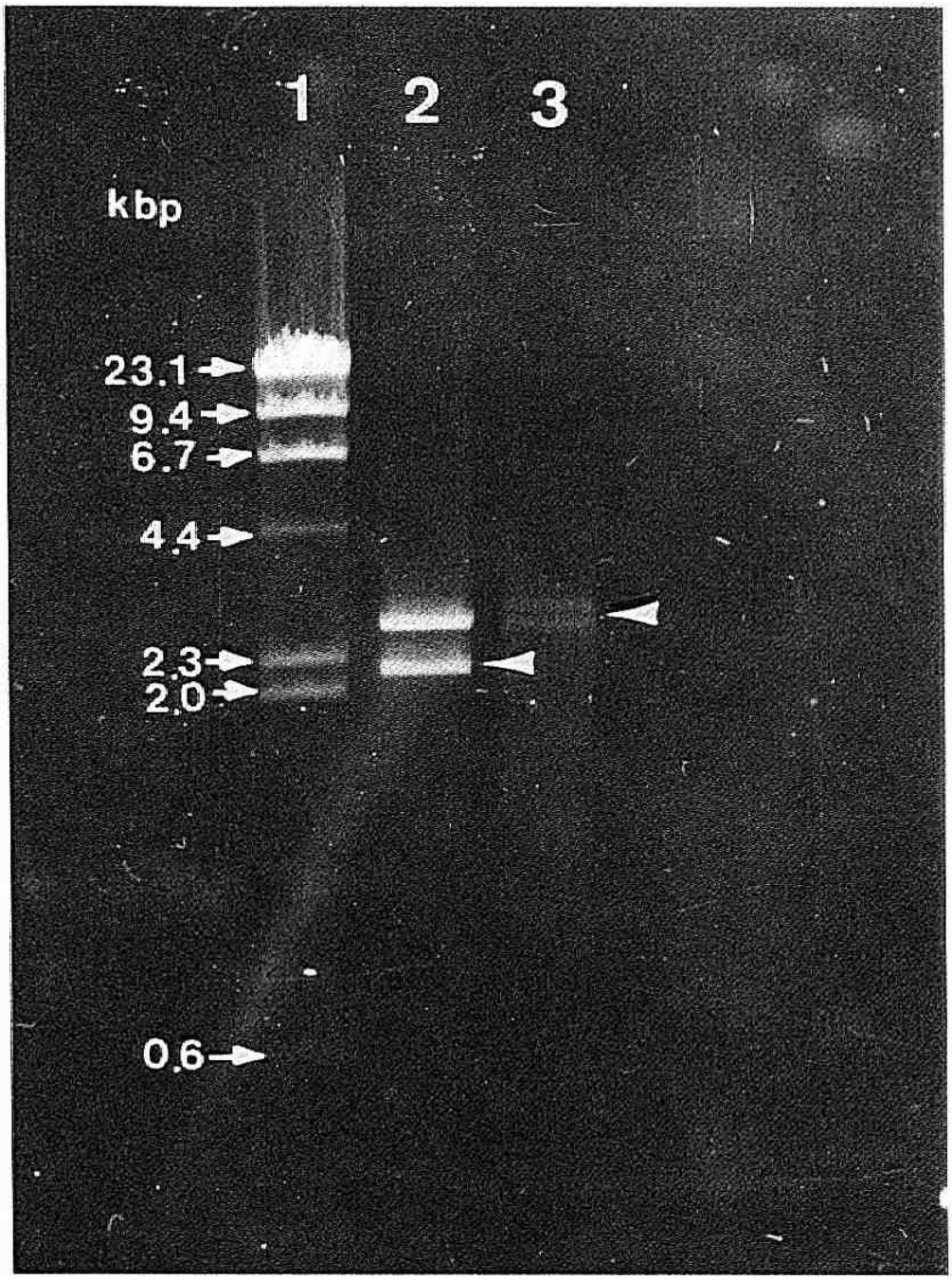

Fig. 1.

*Hin*dIII digested pPG97 and pBFl69 hybrid plasmid DNA on a 0.7% agarose gel. Lane 1: *Hin*dIII digested lambda DNA as molecular size markers. Lane 2: insert DNA from *P. gingivalis* was 2.2 kb. Lane 3: insert DNA from *B. forsythus* was 2.8 kb. Arrow indicates the insert fragment in each of the hybrid plasmids.

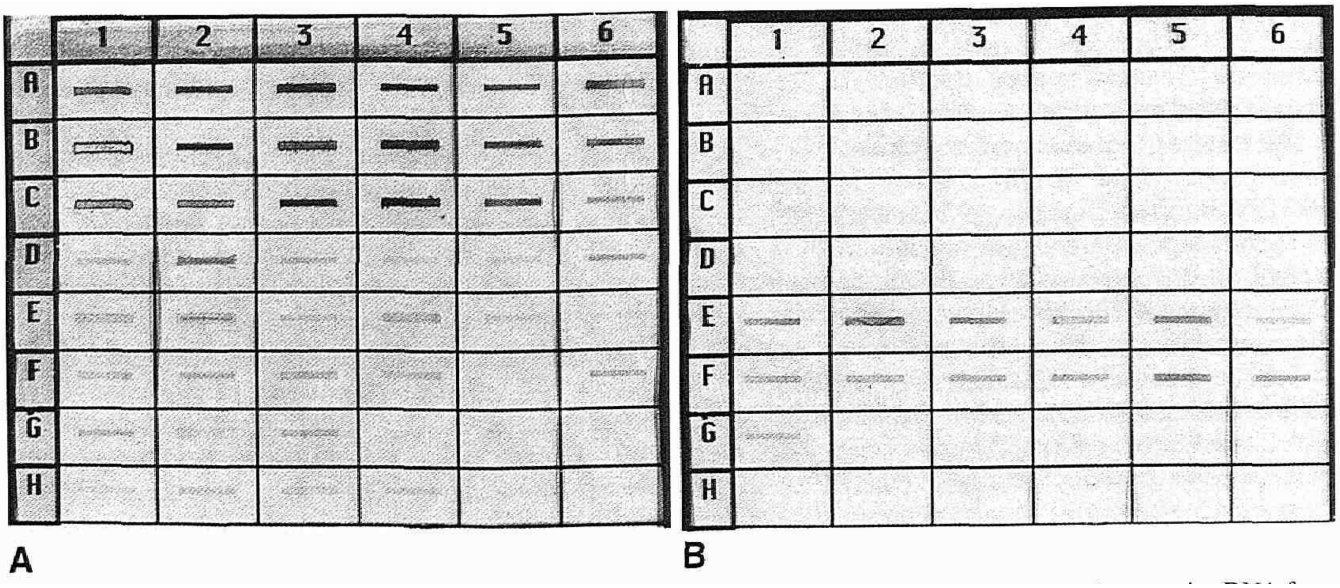

Fig. 2.

Fig. 2A. Digoxigenin-labeled *P. gingivalis* whole genomic probe tested against various oral species. Each slot contains DNA from different species. Slots A1–A6: *P. gingivalis* 33277, LL 8122. HG 66, 7436, HG405, FDC381. Slots B1–B6: *P. gingivalis* USC 1469, USC 1225, USC 1065, USC 1231, USC 1045, USC 2357. Slots C1–C6: *P. gingivalis* USC 1109, M90-863, M90-263, M90-1081, M90-920, M90-1279. Slots D1-D6: *B. forsythus* FDC 338, IFA77B2, 42, 2008, BV15, 293. Slots E1-E6: *P. buccae, P, denticola, P. intermedia, P. nigrescens, P. loescheii, P. melaninogenica*. Slots F1-F6: *P. oralis, P. oris, P. asaccharolytica, B. capillosus, B. gracilis, B. heparinolytica*. Slots G1-G6: *B. oulorum, B. thetaiotaomicron, B. zoogleoformans. C. concisus, C. curvus, C. rectus*. Slots H1-H6: A. *a*. JP2, *C. gingivalis, C. ochracea. C. sputigena, E. corrodens, P. micros. Fig. 2B*. Digoxigenin-labeled cloned pPG97 probe tested against various oral species. Each slot contains DNA from different species. Slots A1–A6: *B. forsythus* FDC 338. IFA 77B2, 42, 2008, BV 15, 293. Slots B1-B6: *B. thetaiotaomicron, C. gingivalis, C. sputigena, C. ochracea, E. corrodens* 470S. 23834. Slots C1-C6: *E. corrodens* 373, *S. flueggei, S. noxia, V. atypica, V. dispar, V. parvula*. Slots D1-D6: *L. buccalis, P. micros, T. pectinovorum, T. denticola, T. vincentii, T. socranskii*. Slots E1-E6: *P. gingivalis* 33277, USC 1469, USC 1225, USC 1065, USC 1231, USC 1045. Slots F1-F6: *P. gingivalis* USC 2357, USC 1109, M90-863, M90-263, M90-1081, M90-920. Slots G1-G6: *P. gingivalis* M90-1279, A. *a.* JP2, Y4, *H. aphrophilus. A. naeslundii, A. viscosus*. Slots H1–H6: *F. nucleatum* subsp. *polymorphum, F. nucleatum* subsp. *nucleatum, F. periodonticum, F. nucleatum* subsp. *vincentii* EM 48, *F. mortiforum, F. nucleatum* subsp. *vincentii* FDC 364.

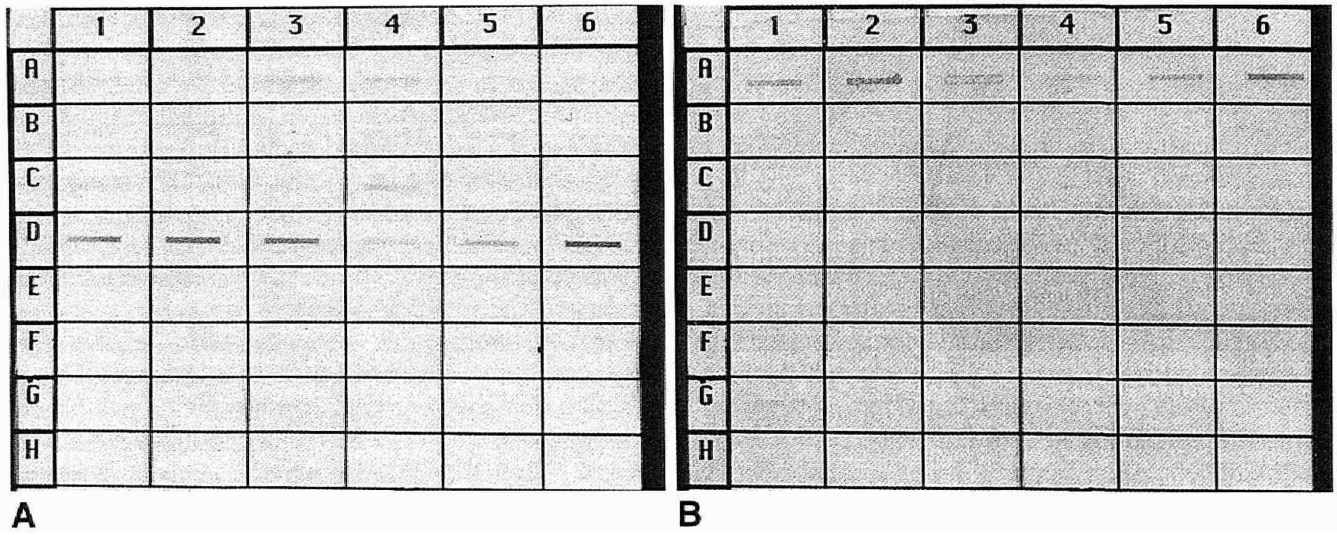

Fig. 3.

Fig. 3A. Digoxigenin-labeled *B. forsythus* whole genomic probe tested against various oral species. Each slot contains DNA from different species. Slots A1–A6: *P. gingivalis* 33277, LL 8122, HG 66, 7436, HG405. FDC381. Slots B1-B6: *P. gingivalis* USC 1469, USC 1225, USC 1065, USC 1231, USC 1045, USC 2357. Slots C1-C6: *P. gingivalis* USC 1109, M90-863, M90-263, M90-1081, M90-920, M90-1279. Slots D1-D6: *B. forsythus* FDC 338, IFA77B2, 42, 2008, BV15, 293. Slots E1-E6; *P. buccae, P. denticola, P. intermedia, P. nigrescens, P. loescheii, P. melaninogenica*. Slots F1-F6: *P. oralis, P. asaccharolytica, B. capillosus, B. gracilis, B. heparinolytica*. Slots G1-G6: *B. oulorum, B. thetaiotaomicron, B. zoogleoformans, C. concisus, C. curvus, C. rectus*. Slots H1-H6: A. *a*. JP2, *C. gingivalis, C. ochracea, C. sputigena, E. corrodens, P. micros. Fig. 3B*. Digoxigenin-labeled cloned pBF169 probe tested against various oral species. Each slot contains DNA from different species. Slots A1-A6: *B. forsythus* FDC 338, IFA 77B2, 42, 2008, BV 15, 293. Slots B1-B6: *B. thetaiotaomicron, C. gingivalis, C. sputigena, C. ochracea, E. corrodens* 470S, 23834. slots C1-C6: *E. corrodens* 373. *S. flueggei, S. noxia, V. atypica, V. dispar, V. parvula*. Slots D1-D6: *L. buccalis, P. micros, T. pectinovorum, T. denticola, T. vincentii, T. socranskii*. Slots E1-E6: *P. gingivalis* 33277, USC 1469, USC 1225, USC 1065, USC 1231, USC 1045. Slots F1-F6: *P. gingivalis* USC 2357, USC 1109, M90-863, M90-263, M90-1081, M90-920. Slots G1-G6: *P. gingivalis* M90-1279, A. *a*. JP2, Y4. *H. aphrophilus, A. naeslundii, A. viscosus*. Slots H1-H6: *F. nucleatum* subsp. *polymorphum. F. nucleatum* subsp. *nucleatum, F. periodonticum, F. nucleatum* subsp. *vincentii* EM 48. *F. mortiforum. F. nucleatum* subsp. *vincentii* FDC 364.

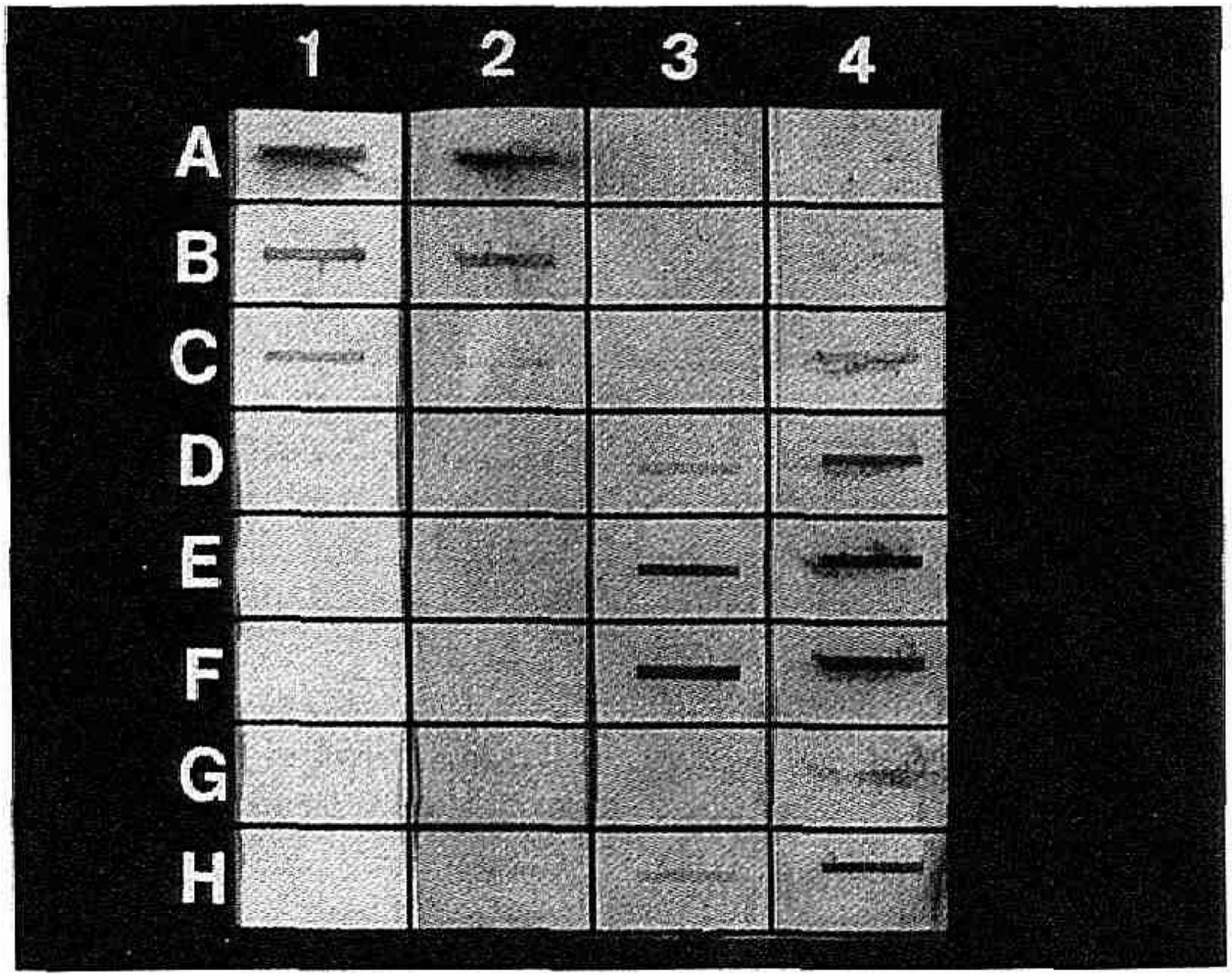

Fig. 4.

Sensitivity of digoxigenin-labeled pPG97 probe and *P. gingivalis* whole genomic probe. Columns 1, 2: 10-fold serially diluted pure cultured *P. gingivalis* (*P.g.*) cells. From top to bottom, 10^8 to 10^1 cells. Columns 3, 4: 10-fold serially diluted concentrations of chromosomal DNA from *P. gingivalis*. From top to bottom, concentration from 10 pg to 1 μg. Columns 1, 4: hybridization with whole genomic probe, column 2. 3: hybridization with pPG97 probe. Negative control: *B. forsythus (B.f.)* chromosomal DNA (Slots H3, H4).

Table 1

Specificity of DNA probes for P. gingivalis and B. forsythus[a]

		DNA probe				
Source of DNA[b]		**whole genomic *P. gingivalis***	**pPG97**	**whole genomic *B. forsythus***	**pBF169**	**pUC19**
pUC19		−	−	−	−	+++
Gram negative species						
Actinobacillus						
actinomycetemcomitans	*JP2*	±	−	−	−	−
	FDC Y4	*ND*	−	*ND*	−	*ND*
Bacteroides forsythus						
	FDC 338	±	−	+++	+++	−
	FDC IFA 77B2	+	−	+++	+++	−
	FDC 42	−	−	+++	++	−
	FDC 2008	−	−	++	++	−
	FDC BV 15	−	−	++	+++	−
	FDC 293	+	−	+++	+++	−
Bacteroides						
capillosus	ATCC 29799	±	−	−	−	−
gracilis	ATCC 33236	−	−	−	−	−
oulorum	ATCC 43324	±	−	−	−	−
thetaiotaomicron	ATCC 29741	−	−	−	−	−
zoogleoformans	ATCC 33285	±	−	−	−	−
Campylobacter						
concisus	FDC 484	−	−	−	−	−
curvus	FDC 640	±	−	−	−	−
rectus	FDC 1219	±	−	−	−	−
Capnocytophaga						
gingivalis	FDC 27	±	±	−	−	−

Source of DNA[b]		DNA probe				
		whole genomic *P. gingivalis*	pPG97	whole genomic *B. forsythus*	pBF169	pUC19
ochracea	FDC 25	±	±	–	–	–
sputigena	FDC 4	±	–	–	–	–
Centipeda periodontii						
	LL 2383	–	–	–	–	–
	LL 2021	–	–	–	–	–
	LL 2441	ND	–	ND	–	ND
Eikenella corrodens						
	ATCC 23834	ND	–	ND	–	–
	FDC 470S	–	–	–	–	–
	FDC 1006	ND	–	ND	–	ND
	FDC 373	ND	±	ND	–	ND
Fusobacterium nucleatum						
subsp. *polymorphum*	ATCC 10953	–	–	–	–	–
subsp. *nucleatum*	ATCC 25586	–	–	–	–	–
subsp. *vincentii*	EM 48	–	–	–	–	–
	FDC 364	–	–	–	–	–
mortiferum	ATCC 25557	–	–	–	–	–
periodonticum	ATCC 33693	–	–	–	–	–
Haemophilus aphrophilus	ATCC 19415	ND	–	ND	–	ND
Leptotrichia buccalis	ATCC 19616	–	–	–	–	–
Prevotella						
buccae	ATCC 33574	±	–	–	–	–
denticola	ATCC 33185	+	–	–	–	–
intermedia	ATCC 25611	–	–	–	–	–
heparinolytica	ATCC 35895	+	–	–	–	–
Prevotella						
nigrescens	ATCC 335663	+	–	–	–	–

		DNA probe				
Source of DNA[b]		**whole genomic *P. gingivalis***	**pPG97**	**whole genomic *B. forsythus***	**pBF169**	**pUC19**
loescheii	ATCC 15930	+	−	−	−	−
melaninogenica	ATCC 25845	±	−	−	−	−
melaninogenica	FDC 379	ND	−	ND	−	ND
oralis	ATCC 33269	±	−	−	−	−
oris	ATCC 33573	−	−	−	−	−
Porphyromonas						
asaccharolytica	ATCC 25260	±	ND	−	ND	−
gingivalis	ATCC 33277	+++	+++	±	−	−
	FDC 381	+++	+++	−	−	−
	HG66	+++	+++	+	−	−
	HG 405	+++	+++	−	−	−
	HG 7436	+++	+++	+	−	−
	LL 8122	+++	+++	−	−	−
	USC 1469	+++	+++	−	−	−
	USC 1225	+++	+++	+	−	−
	USC 1065	+++	+++	±	−	−
	USC 1231	+++	+++	±	−	−
	USC 1045	+++	+++	±	−	−
	USC 2357	+++	+++	±	−	−
	USC 1109	+++	+++	±	−	−
	M90-863	+++	+++	±	−	−
	M90-263	+++	+++	−	−	−
	M90-108I	+++	+++	+	−	−
	M90-920	+++	+++	−	−	−
	M90-1279	+++	+++	−	−	−
Selenomonas						
artemidis	ATCC 43528	−	−	−	−	−

		DNA probe				
Source of DNA[b]		**whole genomic *P. gingivalis***	**pPG97**	**whole genomic *B. forsythus***	**pBF169**	**pUC19**
diane	ATCC 43527	−	−	−	−	−
flueggei	FDC 1304	−	−	−	−	−
noxia	ATCC 43541	−	−	−	−	−
sputigena	ATCC 35185	−	−	−	−	−
Treponema						
denticola	ATCC 35405	−	−	−	−	−
pectinovorum	ATCC 33768	−	−	−	−	−
socranskii	ATCC 35534	−	−	−	−	−
vincentii	N 9	−	−	−	−	−
Veillonella						
atypica	ATCC 27215	−	−	−	−	−
dispar	ATCC 17745	−	−	−	−	−
parvula	ATCC 10790	−	−	−	−	−
Gram-positive species						
Actinomyces						
naeslundii	ATCC 12104	−	−	−	−	−
viscosus	FDC T14	−	−	−	−	−
Pentostreptococcus micros	ATCC 33270	−	−	−	−	−

[a] One μg of chromosomal DNA was applied to each well of the slot blot apparatus. Hybridization reactions were recorded as: − negative, ± faint, + weak, ++ moderate, + + + strong, ND, Not done.

[b] ATCC, American Type Culture Collection; FDC, Forsyth Dental Center; LL, Lai and Listgarten; HG, State University of New York at Buffalo; USC, University of Southern California.