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Keywords

Actinobacillus actinomycetemcomitans, DNA fingerprinting, polymerase chain reaction, restriction fragment length polymorphism, DNA probe, Southern blot

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Evaluating two methods for fingerprinting genomes of *Actinobacillus actinomycetemcomitans*

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Abstract

The arbitrary primer polymerase chain reaction (AP-PCR) and Southern blot restriction fragment length polymorphism (RFLP) were used to genotype the periodontal pathogen *A. actinomycetemcomitans.* Total genomic DNA from 73 strains was extracted by conventional methods. Three random-sequence 10-base oligonucleotide primers were chosen for AP-PCR. The amplified DNA products were separated electrophoretically in a 1% agarose gel containing ethidium bromide and the banding patterns were compared among different strains. For RFLP analysis, DNA was digested with *Eco*RI, separated on a 0.8% agarose gel and transferred to a nylon membrane. The membrane was probed with a previously characterized 5.2 kilobases (kb) DNA fragment cloned from *A. actinomycetemcomitans* strain Y4. The probe was labeled with digoxigenin, and hybridized fragments were detected with anti-digoxigenin antibody. AP-PCR produced 4–10 DNA bands in the 0.5–5 kb regions and distinguished 9, 13 or 17 genotypes, depending on the specific primer used. Southern blot RFLP analysis revealed 12 hybridization patterns consisting of 1 or 2 DNA fragments (2–23 kb). The addition of the Southern blot analysis to the AP-PCR analysis gave rise to a total of 30 DNA profiles among the 73 *A. actinomycetemcomitans* study strains. The results indicate that both AP-PCR and Southern blot analysis are useful in clonal analysis of *A. actinomycetemcomitans.*

Keywords

Actinobacillus actinomycetemcomitans; DNA fingerprinting; polymerase chain reaction; restriction fragment length polymorphism; DNA probe; Southern blot

Actinobacillus actinomycetemcomitans seems to be the primary etiological agent in localized juvenile periodontitis and some cases of adult periodontitis (22). The organism can also cause serious nonoral infections (19).

Evidence suggests a clonal nature of *A. actinomycetemcomitans* in oral and nonoral infections. Zambon et al. (29) detected *A. actinomycetemcomitans* serotype b more frequently than serotype a and c in localized juvenile periodontitis patients in the United States. Asikainen et al. (2) demonstrated a predominance of serotype b in periodontitis patients and of serotype c in healthy individuals in Finland. In contrast, serotype c seems to be responsible for many nonoral *A. actinomycetemcomitans* infections (28). Zambon et al. (30) showed that most *A. actinomycetemcomitans* strains from periodontitis lesions

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elaborated a potent leukotoxin, whereas only a few *A. actinomycetemcomitans* isolates from periodontally healthy subjects released detectable levels of leukotoxin. Preus et al. (18) proposed that phage-infected *A. actinomycetemcomitans* strains were more virulent than noninfected strains. Using restriction fragment length polymorphism (RFLP) typing, DiRienzo & Slots (8) suggested that *A. actinomycetemcomitans* RFLP type B, corresponding to *A. actinomycetemcomitans* strain JP2, was particularly virulent, as judged from its presence in 3 subjects who converted from periodontal health to localized juvenile periodontitis.

Identifying *A. actinomycetemcomitans* clones associated with progressive periodontitis or nonoral infections will be critical for understanding the pathogenic mechanism of this organism. Clonal analysis may also help delineate the mode of transmission of *A. actinomycetemcomitans* from subject to subject. Knowledge of transmission determinants may be exploited in the prevention and treatment of periodontal *A. actinomycetemcomitans* infection. Further, clonal analysis of *A. actinomycetemcomitans* isolates from oral and nonoral sites within the same subject might provide insight into the question of whether oral *A. actinomycetemcomitans* is a reservoir for nonoral infections.

Polymerase chain reaction (PCR) is an important analytic method in molecular genetics. It has the ability to amplify DNA by using specific primers to known DNA sequences of the target (4, 9). It has been adopted to identify infectious agents in biological samples (4, 5, 9, 16). The extremely high sensitivity and specificity of the PCR technique allows the detection of as little as 10 target bacterial cells among 10^6 microorganisms (10, 17). More recently, arbitrarily primed PCR (AP-PCR) or randomly amplified polymorphic DNA (RAPD) has been used for detecting genetic polymorphism of both eukaryotic and procaryotic cells (25–27). AP-PCR has the advantage that no prior knowledge of the DNA sequence is required and that it is readily performed. It employs short oligonucleotides (10–12 bases) of random sequences. The resulting amplification products, consisting of various DNA fragments, may serve as polymorphic markers. AP-PCR has been used for genetic analysis of the genus *Candida* (11), for DNA fingerprinting of *Haemophilus somnus* (14), for generating polymorphism markers linked to specific genes or genomic regions in plants (13), for the study of population genetics (6) and for gene mapping (15, 20).

The aim of the present study was to determine the utility of AP-PCR for clonal analysis of *A. actinomycetemcomitans* clinical isolates and to compare the results with those of an established Southern blot analysis using a cloned *A. actinomycetemcomitans* DNA fragment.

Material and methods

Bacterial strains and culture conditions

A. actinomycetemcomitans strains ATCC 29522, ATCC 29523 and ATCC 29524 were obtained from the American Type Culture Collection, Rockville, MD, strain NCTC 9710^T from the National Collection of Type Cultures, London, UK, strain Y4 from S.S. Socransky, Forsyth Dental Center, Boston, MA and strain JP2 from N. S. Taichman, University of Pennsylvania School of Dental Medicine, Philadelphia, PA. The remaining 67 *A. actinomycetemcomitans* strains were fresh clinical isolates from successive periodontal specimens submitted by extramural dentists to the Oral Microbiology Testing Laboratory at the University of Southern California School of Dentistry. Each positive patient provided one *A. actinomycetemcomitans* isolate. The periodontal specimens were obtained by paper points, transported in 2 ml of VMGA III medium and plated onto TSBV selective medium, which was incubated for 4 days at 35°C in 10% CO_2 in air (23). *A. actinomycetemcomitans* was identified on the basis of agar adherent colony with internal star morphology, gram-

negative coccobacillary rods, strong positive catalase reaction, and negative MUG reaction (1). A single *A. actinomycetemcomitans* colony was subcultured to TSBV medium to obtain confluent growth for DNA extraction.

DNA extraction

DNA from *A. actinomycetemcomitans* strains was extracted essentially as described by Smith et al. (24). Briefly, bacterial cells were harvested from a TSBV agar plate and lysed at 37°C for 60 min in a solution of 600 μl of TE buffer (10 mM Tris-HCl, pH 7.6, 1 mM EDTA), 0.5% sodium dodecyl sulfate (SDS) and 0.01% proteinase K. The lysate was mixed with 150 μl of 5 M NaCl and 85 μl of CTAB solution (10% hexadecyltrimethyl ammonium bromide in 0.7 M NaCl) at 65°C for 20 min. Proteins and polysaccharides were precipitated with an equal volume of chloroform:isoamyl alcohol (24:1, v/v). After centrifugation at 12,000 ×*g* for 15 min, the supernatant was mixed with equal volumes of saturated phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v). After centrifugation, DNA in the supernatant was precipitated with 0.6 volumes of isopropanol, harvested by centrifugation, redissolved in 300 μl of TE containing 2 μl of R Nase A (10 mg/ml), incubated at 50°C for 60 min, and re-extracted in 30 μl of 3 M sodium acetate buffer (pH 7.6) and chloroform:isoamyl alcohol. DNA in the aqueous phase was precipitated with 2 volumes of ice-cold 95% ethanol, collected by centrifugation, and washed once with 70% ethanol and, after removal of the ethanol, allowed to dissolve overnight in 200 μl of TE buffer at 35°C. The DNA solution was stored at − 20°C.

DNA probe construction

A DNA fragment of the length 5.2 kilo-bases (kb) from *A. actinomycetemcomitans* strain Y4 was cloned into the pBR 328. The plasmid was digoxigenin-labeled using the Genius™ Nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). One μg of the plasmid was heat denatured for 10 min in boiling water, quickly cooled on ice, and incubated for 24 h at 37°C with 5 μl of digoxigenin labeling mixture containing dCTP, dATP, dGTP (1 mmol/l each), dTTP (0.65 mmol/l), digoxigenin-dUTP (0.35 mmol/l), 5 μl of a random hexanucleotide mixture, 2.5 μl of Klenow fragments and sterile water up to 50 μl. The labeling reaction was stopped with 5 μl of 0.2 M EDTA (pH 8.0). Labeled probes were precipitated with 5 μl of 4 M LiCl and 150 μl of ice-cold 95% ethanol at −20°C for at least 2 h, centrifuged at 12,000 × g for 10 min, washed in 70% ethanol and dried under vacuum. The pellet was dissolved in 500 μl of TE and stored at − 20°C.

Southern blot

For Southern blot analysis, 2 μg each of the DNA sample was digested with 40 units of *Eco*RI restriction endo-nuclease (Boehringer Mannheim Biochemicals) for at least 2 h at 37°C. The digested DNA samples were separated on 0.8% agarose gel in 0.04 M Tris-acetate and 0.001 M EDTA (1 ×TAE buffer) containing 0.5 μg/ml ethidium bromide for 3 h at 50 V using a Minnie Submarine agarose gel unit (Hoefer Scientific Instruments, San Francisco, CA). For each gel, the digoxigenin-labeled lambda DNA, digested to completion with *Hin*dIII (molecular weight marker II, Boehringer Mannheim Biochemicals), was electrophoresed parallel with the DNA samples to provide a molecular size marker. The gels were then treated with 0.2 N HC1 for 10 min and rinsed in running water for 10 min, and the DNA fragments were transferred overnight to nylon membranes (Hybond N +, Amersham Corp., Arlington Heights, IL) using 0.4 N NaOH as the transfer solution. The membranes were baked for 30 min at 120°C, rehydrated with 2 × SSC (1×SSC = 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) and incubated at 68°C for 2–4 h in a pre-hybridization solution containing 5 × SSC, 1% blocking reagent (Boehringer Mannheim Biochemicals), 0.1% N lauryl sarcosine and 0.02% SDS. Heat-denuatured digoxigenin-

labeled DNA probe was then added into the pre-hybridization solution to a final concentration of 100–200 ng/ml. Hybridization was performed at 68°C overnight in a shaking water bath. Following hybridization, the blots were washed twice with 2 × SSC and 0.1% SDS at room temperature for 5 min each, and twice with 0.1 × SSC and 0.1% SDS at 65°C for 15 min each.

Detection of DNA probe

Immunological detection of the digoxigenin-labeled probe and target DNA complex was performed according to the manufacturer's suggestion. Briefly, the membranes were equilibrated in 100 mM Tris-HCl (pH 7.5) and 150 mM NaCl (Buffer I) for 5 min and incubated in Buffer I containing 1% blocking reagent (Buffer II) for 30 min at room temperature. An anti-digoxigenin antibody conjugated with alkaline phosphatase diluted 1:10.000 in Buffer II was added to the membranes and incubated for 30 in in at room temperature. The membranes were washed twice in Buffer I for 15 min each at room temperature and then equilibrated with 100 mM Tris-HCl (pH 9.5), 100 mM NaCl and 50 mM $MgCl_2$ (Buffer III) for 2 min. Colorimetric development was performed in the dark for 1–2 h with 10 ml of Buffer III containing 45 μl 4-Nitro Blue Tetrazolium chloride (NBT, 75 mg/ml) and 35 μl 5-bromo-4-chloro-3-indolyl phosphate (X-phosphate, 50 mg/ml). The reaction was stopped by rinsing in TE, pH 8.0.

PCR primers

Twenty 10-base oligonucleotide primers were obtained from Operon Technologies, Inc., Alameda, CA. The sequences of the primers were selected randomly, with the requirement that their guanine-cytosine content was 60% to 70% and that they had no self-complementary ends. The base sequences (5′–3′) of the 20 primers were CAGGCCCTTC, TGCCGAGCTG, A-GTCAGCCAC, AATCGGGCTG, A-GGGGTCTTG, GGTCCCTGAC, GA-AACGGGTG, GTGACGTAGG, GG-GTAACGCC, GTGATCGCAG, CAA-TCGCCTGT, TCGGCGATAG, CA-GCACCCAC, TCTGTGCTGG, TTC-CGAACCC, AGCCAGCGAA, GAC-CGCTTGT, AGGTGACCGT, CAA-ACGTCGG and GTTGCGATCC. A preliminary screening of the 20 primers showed that the 3 primers AGTCAG-CCAC (OPA-03), GAAACGGGTG (OPA-07), CAGCACCCAC (OPA-13) could be used to distinguish individual *A. actinomycetemcomitans* strains, and these 3 primers were used in this study.

DNA amplification

Amplification reactions were performed in volumes of 100 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM $MgCl_2$, 0.1 mM each of dNTP (Pharmacia LKB Biotechnology, Piscataway, NJ), 2.5 U Taq DNA polymerase (Perk-in Elmer Cetus, Norwalk, CT), 0.2 μM primer and 0.2 μg genomic DNA. The reaction buffer was overlaid with mineral oil and amplified using a PTC-100 thermal controller (MJ Research, Watertown, MA). Since very short (10-base) primers with less specificity than longer primers were used, the method is quite sensitive to small variations in the temperature cycle, particularly the annealing temperature. The temperature profile for amplification was 94°C for 5 min for initial denaturation, and 2 cycles of 94°C for 1 min, 36°C for 2 min, 72°C for 2 min, followed by 35 cycles of 94°C for 1 min, 42°C for 2 min and 72°C for 2 min. After the last cycle, the amplified products were extended for another 10 min at 72°C. Twenty μl of the amplified products was analyzed through 1% agarose gel in 1 × TAE buffer using the Minnie Submarine agarose gel unit at 50 V for 3 h and detected by staining with elhidium bromide. One μg of 1 kb DNA ladder (GIBCO BRL Life Technologies, Gaithersburg, MD) was run parallel with the DNA as molecular size markers.

Results

In preliminary studies, 20 random primers (OPA-01 to OPA-20) were screened for their utility in the AP-PCR clonal analysis of *A. acttnomycetemcomitans.* The same primer and strain produced reproducible DNA banding profiles of the amplification products. However, different primers yielded very different banding profiles, ranging from a few faint bands to several marked bands, which allowed for discrimination among strains. Three primers, OPA-03, OPA-07 and OPA-13, which yielded 4–10 DNA bands with molecular sizes between 0.5–5 kb, were selected for the analysis of 73 *A. actinomycetemcomitans* study strains.

The OPA-03 primer resulted in 14 (Fig. 1A), OPA-07 in 9 (Fig. 1B), and OPA-13 in 17 (Fig. 1C) AP-PCR profiles. In general, strains with similar or identical DNA fingerprints were easily recognized visually (Fig. 2). The presence of minor bands in DNA fingerprints permitted further distinction among strains with the same general AP-PCR profile (for example Fig. 2, lane 8 and 9). An arbitrary number was assigned to each AP-PCR profile, from 1 to 14 for OPA-03, 1 to 9 for OPA-07 and 1 to 17 for OPA-13. Some strains were assigned to the same AP-PCR profile group whether OPA-03, OPA-07 or OPA-13 was used for amplification (strains belonging to 1-1-1, 6-6-6 and 7-7-7; Table 1). Other strains were placed in the same AP-PCR profile group using two of the primers, but were further divided into two profile groups by the third primer (for example profile 5-5-5 and 10-5-5; Table 1).

Fig. 3 shows Southern blot analysis of *A. actinomycetemcomitans* strains performed with a 5.2 kb cloned DNA probe derived from *A. actinomycetemcomitans* strain Y4. The DNA probe hybridized to one or two fragments (molecular size ranging from 2.3–23 kb) in *Eco*RI-restricted *A. actinomycetemcomitans* genomic DNA. The resulting profile was unambiguous and categorized the 73 strains into 12 distinguishable hybridization patterns.

Table 1 summarizes the AP-PCR and Southern blot analysis of the 73 *A. actinomycetemcomitans* strains tested. AP-PCR analysis using the selected 3 primers distinguished 21 *A. actinomycetemcomitans* profiles among 73 strains. The addition of the results from Southern blot analysis of the AP-PCR analysis gave rise to a total of 30 DNA profiles. The AP-PCR profile 4-4-4 represented 17 of the 73 test strains (23.3%), including the reference strain ATCC 29523. The profile 3-3-3 constituted 14 strains (19.2%), including the type strain NCTC 9710^{T}. The profile 2-2-2 comprised 7 strains (9.6%), including strain JP2. The profile 1-1-1 made up 5 strains (6.8%), including the reference strain ATCC 29522 and strain Y4. The profile 5-5-5 included 5 strains (6.8%) and the profile 8-8-8 included 4 strains (5.5%). Each of the remaining 15 AP-PCR profiles comprised less than 5% of total test strains. The most common DNA probe profiles were profile 4 (20 strains, 27.4% of total strains), profile 3 (12 strains, 16.4%), profile 2 (9 strains, 12.3%) and profile 1 (5 strains, 6.8%).

Discussion

This study examined the utility of 2 DNA methods for clonal analysis of *A. actinomycetemcomitans* strains. Depending on the chosen arbitrary primers, the AP-PCR examination of the 73 study strains resulted in 9, 14 or 17 amplification profiles. The same 73 strains were classified into 12 different profiles by Southern blot analysis using a randomly cloned 5.2 kb DNA probe from *A. actinomycetemcomitans* strain Y4. The combination of AP-PCR and Southern blot analyses classified the 73 study strains into 30 distinct groups. The results indicate that both methods individually or in combination may have applicability in the clonal analysis of *A. actinomycetemcomitans.* AP-PCR has the advantage in having a wide selection of primers for DNA fingerprinting and in being easier

to perform than Southern blot analysis. However, since the PCR method is sensitive to variation in temperature profiles and to changes in concentrations of genomic DNA, primers, nucleotides and $MgCl_2$, the AP-PCR clonal analysis requires a careful control of all variables. The advantage of Southern blot analysis is the simplicity of the hybridization patterns generated, which allow unambiguous genotype classification of individual *A. actinomycetemcomitans* strains.

AP-PCR has been used to DNA-fingerprint *Porphyromonas gingivalis* (12, 21) and strains of medically important bacterial species (3, 11, 14). AP-PCR was able to distinguish *H. somnus* from other bacterial species and to identify individual *H. somnus* strains (14). Several virulent *H. somnus* strains were found to possess nearly identical AP-PCR DNA fingerprints, suggesting a clonal origin of virulent strains within this species. In addition, the genetic relatedness between *H. somnus* strains could be determined from the similarity coefficient calculated from pairwise comparison of the DNA fingerprints generated by AP-PCR (14). AP-PCR DNA fingerprinting of the *Candida* genus revealed a DNA profile unique for individual strains (11). However, the AP-PCR DNA fingerprints of strains from the same *Candida* species were similar and may be used for species identification (11). The AP-PCR method has also been used to identify individual strains of the *Aspergillus fumigatus* species (3). Similarly, in the present study, we observed that AP-PCR DNA fingerprinting may be used to discriminate individual *A. actinomycetemcomitans* strains.

The *A. actinomycetemcomitans* clinical isolates originated from samples submitted by extramural dentists from very diverse geographic regions of the United States and, most likely, were from epidemiologically independent sources. The observation that some strains from diverse sources exhibited identical AP-PCR DNA fingerprints may suggest the clonal nature of the species *A. actinomycetemcomitans.* However, we have no information on the disease activity of the periodontal sites samples, and consequently could not determine whether certain AP-PCR profiles represent virulent *A. actinomycetemcomitans* clones.

A. actinomycetemcomitans strains with the same general AP-PCR profile may be distinguished by minor DNA bands or by using different primers in AP-PCR. In addition, we have observed that bacterial DNA fingerprints generated from 2 arbitrary primers used simultaneously are different from the composite of DNA fingerprints from the 2 primers used singularly (unpublished data). Therefore, the AP-PCR DNA fingerprinting method has available a virtually limitless selection of possible primers, from single primers to various combinations of multiple primers. This suggests that AP-PCR can be very useful in strain identification in studies on *A. actinomycetemcomitcms* transmission.

The diversity in the DNA fingerprints generated by Southern blot depends on the heterogeneity of the restriction enzyme sites in the *A. actinomycetemcomitans* genome containing the 5.2 kb fragment (or fragment with homology to the 5.2 kb probe). A similar cloned DNA probe was used in previous studies for clonal analysis of *A. actinomycetemcomitans* and for verification of transmission of this organism among family members (7, 8). In one of the studies by DiRienzo & Slots (8), 6 RFLP profiles were recognized among 133 *A. actinomycetemcomitans* isolates obtained from 12 subjects. In addition, it was suggested that RFLP profile type B may represent a pathogenic *A. actinomycetemcomitans* clone (8). Since this study included 73 strains from epidemiologically independent sources, a higher number of DNA probe profiles was detected. The profile 2 in the present Southern blot analysis, corresponding to the RFLP type B (8), contained 9 strains.

In conclusion, this study showed that both AP-PCR and Southern blot analyses are useful in the clonal analysis of *A. actinomycetemcomitans* strains. The DNA fingerprints generated

by the two methods can separate clinical *A. actinomycetemcomitans* strains into a large number of genetic groups. These DNA fingerprinting methods will have utility in determining the clonality of *A. actinomycetemcomitans* with respect to virulence and the transmission of this organism among family members and other exposed individuals.

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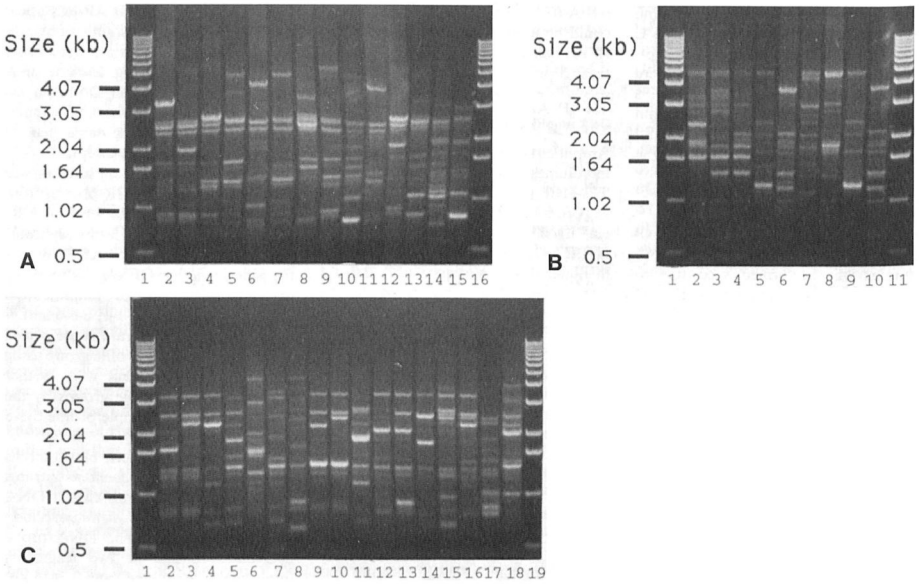

Fig. 1.

The representative *A. actinomycetemcomitans* AP-PCR profiles using a single random sequence primer. Two hundred nM of primer was used to amplify 200 ng of *A. actinomycetemcomitans* genomic DNA by PCR as described in Material and methods. The amplification products were separated on a 1% agarose gel containing ethidium bromide. The DNA banding profiles were examined visually under ultraviolet illumination. **A**. 14 representative *A. actinomycetemcomitans* profiles using OPA-03. Lane 1 and 16, molecular size markers; lane 2, strain Y4: lane 3, JP 2; lane 4, NCTC 9710^T; lane 5, strain 91; lane 6, strain 621; lane 7, strain 29; lane 8, strain 266; lane 9, strain 631; lane 10, strain 361; lane 11, strain 386; lane 12, strain 474; lane 13, strain 606; lane 14, strain 656; lane 15, strain 852. **B**. Nine representative AP-PCR profiles usign OPA-07 as primer. Lane 1 and 11, molecular size markers: lane 2, strain Y4; lane 3 JP2; lane 4, NCTC 9710^T; lane 5, ATCC 29523; lane 6, strain 23; lane 7, strain 29; lane 8, strain 266; lane 9, strain 606;: lane 10, strain 631. **C**. Seventeen representative AP-PCR profiles using OPA-13 as primer. Lane 1 and 19, molecular size markers; lane 2, strain Y4: lane 3, JP2; lane 4, NCTC 9710^T; lane 5, ATCC 29523; lane 6, strain 23; lane 7, strain 29; lane 8, strain 266; lane 9, strain 631; lane 10, strain 681; lane 11, strain 361; lane 12, strain 36; lane 13, ATCC 29524; lane 14, strain 439; lane 15, strain 474; lane 16, strain 480; lane 17, strain 606; lane 18, strain 656.

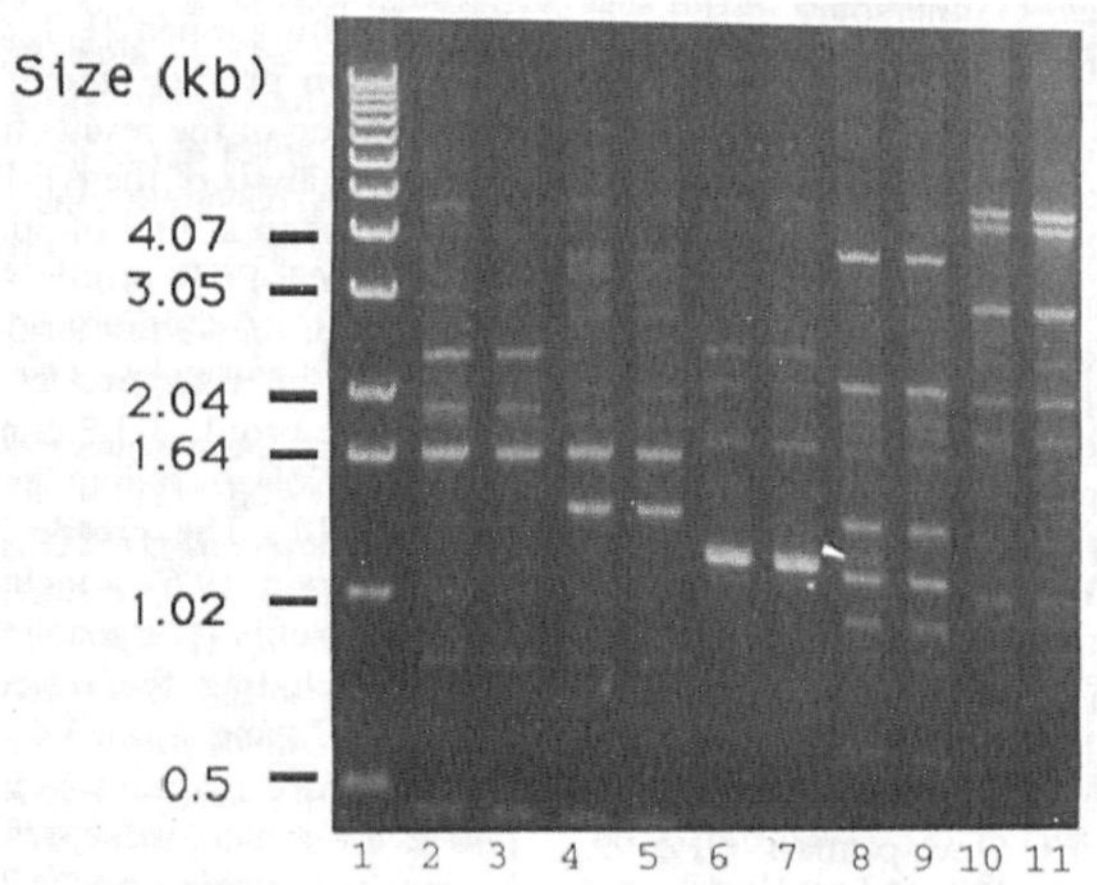

Fig. 2.

Five pairs of *A. actinomycetemcomitans* strains with similar DNA fingerprints generated by AP-PCR using OPA-07 as primer. Lane 1 and 11, molecular markers; lane 2 and 3, Y4 and ATCC 29522; lane 4 and 5, JP 2 and strain 641; lane 6 and 7, ATCC 29523 and strain 91; lane 8 and 9, strain 23 and strain 383; lane 10 and 11, strain 29 and strain 835. Minor DNA bands may further distinguish strains classified under the same AP-PCR profiles. For example, an extra DNA band in the DNA fingerprint (lane 8, indicated by an arrow) may be used to distinguish strain 23 from strain 383.

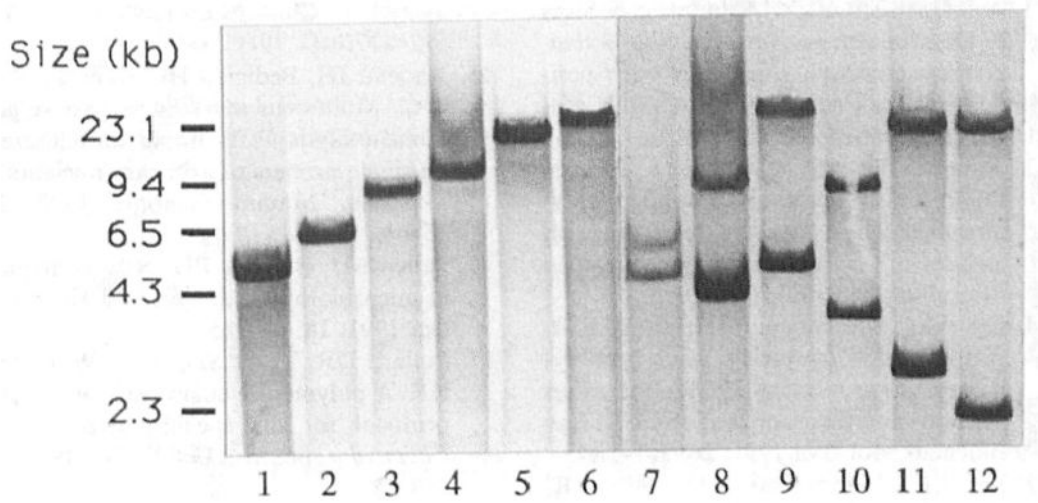

Fig. 3.

Twelve representative *A. actinomycetemcomitans* DNA probe profiles from Southern blot analysis. *A. actinomycetemcomitans* genomic DNA was digested to completion with *Eco*RI and hybridized with digoxigenin-labeled, 5.2 kb cloned DNA from strain Y4. The probe was detected with alkaline phosphatase-conjugated anti-digoxigenin antibody and enzyme substrate. Lane 1, ATCC 29522; lane 2, strain 641; lane 3. NCTC 9710^{T}; lane 4, strain 79; lane 5, strain 681; lane 6, strain 631; lane 7, strain 606; lane 8. strain 474; lane 9, strain 266; lane 10, ATCC 29524; lane 11, strain 283; lane 12, strain 29.

Table 1

AP-PCR and cloned DNA probe for distinguishing 73 *A. actinomycetemcomitans strains*

AP-PCR profile*	DNA probe*	Strains
1-1-1	1	ATCC 29522, Y4 48, 493, 515
2-2-2	2	JP2, 640, 641, 770, 806. 848, 13305
2-8-9	2	2468
2-8-12	10	ATCC 29524
2-8-12	3	649
3-2-3	3	282
3-3-3	3	NCTC 9710^{T}, 97, 126, 761, 3176. 827
3-3-3	5	3, 79, 643. 719, 864. 2490
3-3-11	5	36, 720
3-3-11	8	874
3-3-13	7	439
3-3-15	3	480
4-4-4	4	ATCC 29523, 91, 96, 101, 278, 283. 287, 300. 340, 475, 508, 594, 638, 2484, 13161, 13189. 80034
5-5-5	3	23, 2482
5-5-5	4	476
5-5-5	5	621
5-5-5	7	383
6-6-6	6	29, 835, 889
7-7-7	9	266, 832
8-8-8	7	754, 885
8-8-8	8	631, 632
8-8-9	7	681
8-8-9	8	939
9-4-10	3	361
9-4-10	4	45
10-5-5	5	386
11-8-14	11	474
12-4-10	4	852
13-9-16	12	606
14-8-17	2	656

*Denotes profiles for OPA-03/OPA-07/OPA-13 and DNA probe. Individual profiles were assigned an arbitrary number from 1 to 14 for primer OPA-03, 1 to 9 primer OPA-07. 1 to 17 for primer OPA-13. and 1 to 12 for the DNA probe.