JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1996, p. 2386–2390 Vol. 34, No. 10
0095-1137/96/$04.00+0

DNA Fingerprinting by Infrequent-Restriction-Site Amplification

GERALD H. MAZUREK,[1]* VENKAT REDDY,[1] BARBARA J. MARSTON,[2] WALTER H. HAAS,[3] AND JACK T. CRAWFORD[1]

National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; Division of Infectious Diseases, Emory University School of Medicine, Atlanta, Georgia 30303[2]; and Department of General Pediatrics, University Children's Hospital, Heidelberg University, Heidelberg 69120, Germany[3]

Received 2 April 1996/Returned for modification 10 June 1996/Accepted 21 June 1996

Identification of bacterial strains by DNA fingerprinting facilitates epidemiologic studies and improves disease control. For some species of organisms, no typing method is available; for others, typing methods are tedious. We developed a method of amplifying DNA sequences flanking infrequent restriction sites by PCR and used the method to produce strain-specific electrophoretic patterns from crude bacterial lysates. This method of fingerprinting is rapid, sensitive, and widely applicable. Identical enzymes, adaptors, primers, and PCR conditions were used to characterize 32 *Mycobacterium avium-M. intracellulare* isolates, 4 *Pseudomonas aeruginosa* isolates, and 4 *Staphylococcus aureus* isolates.

Molecular typing techniques are important tools for studying the epidemiology of human, animal, and plant infections. An array of typing methods has been developed. Each typing method attempts to identify unique attributes of a bacterial strain which differentiate it from other strains. The utility of a particular typing method can be judged by assessing its discriminatory powers, range of application, reproducibility, and ease of performance. Restriction fragment length polymorphism analysis has been extremely useful to epidemiologists (12). However, for most bacteria the number of fragments produced by typical restriction enzymes is large, making reliable comparisons difficult. Southern blotting and hybridization with labeled probes reduces the number of visible fragments to a manageable number. However, this approach requires construction of sequence-specific probes, and these probes have a limited range of application. An alternative approach which limits the number of restriction fragments uses infrequently cutting restriction enzymes (4, 11). The large restriction fragments produced are separated by pulsed-field gel electrophoresis. The resulting electrophoretic patterns are highly discriminatory for a wide range of organisms. However, large amounts of high-molecular-weight DNA are needed. Purification of high-molecular-weight DNA in agarose and the electrophoretic separation of the large restriction fragments are time-consuming and tedious.

We developed a method of amplifying DNA sequences flanking infrequent restriction sites and used the method to produce strain-specific electrophoretic patterns from crude bacterial lysates. We refer to the method as infrequent-restriction-site PCR (IRS-PCR) fingerprinting. Identical enzymes, adaptors, primers, and PCR conditions were used to characterize 32 *Mycobacterium avium-M. intracellulare* (MAI) isolates, 4 *Pseudomonas aeruginosa* isolates, and 4 *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

Organisms. Twelve MAI isolates were obtained from the clinical laboratory of the University of Texas Health Center at Tyler (UTHCT). The isolates were recovered from different clinical specimens from five patients, as indicated in Table 1. Twenty additional isolates of MAI from human blood specimens were obtained from clinical laboratories in 20 different cities (Table 2). DNA probes (Accuprobe; GenProbe Inc., San Diego, Calif.) were used to confirm that the isolates were MAI. Four *P. aeruginosa* isolates were obtained from Ali Azghani (Department of Biochemistry, UTHCT) and represented subcultures of three referenced strains from the American Type Culture Collection (ATCC), Rockville, Md., and a clinical isolate from UTHCT, as indicated in Table 2. Four *S. aureus* isolates were obtained from the clinical laboratory of UTHCT and represented three clinical isolates and one referenced strain from ATCC, as indicated in Table 2. MAI isolates were cultured in Middlebrook 7H9 broth supplemented with 10% oleic acid-albumin-dextrose enrichment and 0.1% Tween 80; *Pseudomonas* isolates were cultivated in tryptic soy broth; *S. aureus* isolates were cultured in Mueller-Hinton broth. All media was purchased as powder from Becton Dickinson Microbiology Systems, Cockeysville, Md. The isolates were frozen at −70°C in tryptic soy broth with 15% glycerol until needed for study.

Adaptors and primers. The designations and sequences of the oligonucleotides used are listed in Table 3; their function is schematically depicted in Fig. 1. The oligonucleotides were purchased from Midland Certified Reagent Co., Midland, Tex. Adaptors were constructed to ligate specifically to the CG-3′ two-base overhang generated by *Hha*I digestion or to the 5′-CTAG four-base overhang generated by *Xba*I digestion. The *Hha*I adaptor (AH) consists of a 22-base oligonucleotide (AH1) with a 7-base oligonucleotide (AH2) annealed to bases 14 through 20 from the 5′ end leaving a CG-3′ overhang. The sequence of AH1 is identical to the arbitrary sequence of IS*08-92* used by Haas et al. (3) for mixed-linker PCR fingerprinting of *Mycobacterium tuberculosis*. To prepare the adaptor, AH1 and AH2 were mixed in equal molar amounts in 1× PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% [wt/vol] gelatin) and were allowed to anneal as the mixture cooled from 80 to 4°C over 1 h in a thermal cycler. The stock adaptor was stored at −20°C at a concentration of 14.4 μM. The *Xba*I adaptor (AX) consists of an 18-base oligonucleotide (AX1) with a 7-base oligonucleotide annealed to bases 5 through 11 from the 5′ end leaving a 5′-CTAG overhang. The 5′ end of AX1 was phosphorylated with T4 polynucleotide kinase as specified by the manufacturer (New England Biolabs, Beverly, Mass.). The kinase was subsequently inactivated by heating the mixture to 65°C for 10 min. AX2 and phosphorylated AX1 were mixed and annealed as described above.

An oligonucleotide primer (PX) was constructed to complement AX1 and the one base left on the 3′ end of the native DNA following *Xba*I digestion. Four additional primers (PX-A, PX-C, PX-G, or PX-T) were constructed; their sequences were identical to that of PX except that an additional base (A, C, G, or T, respectively) was placed at their 3′ end. Oligonucleotide AH1 was also used as a primer in PCR.

Preparation of template DNA. Bacterial suspensions (10^5 to 10^8 cells) were lysed in a microtube containing 200 μl of TE buffer (10 mM Tris Cl [pH 7.6], 1 mM EDTA), 200 μl of chloroform, and 100 μl of zirconium beads by vigorous shaking (1). A portion of the lysate (2.5 μl) was digested with 10 U of *Hha*I and 10 U of *Xba*I in 1× NEB4 buffer (final volume, 12.5 μl) for 1 h at 37°C. All enzymes were obtained from New England Biolabs unless stated otherwise. T4 DNA ligase (400 U), ATP (12.6 pmol), 10× ligase buffer (0.75 μl), the *Xba*I adaptor AX (20 pmol), the *Hha*I adaptor AH (20 pmol), and water were added for a total volume of 20 μl. The mixture was incubated at 16°C for 1 h to ligate the adaptors to the digested DNA and then at 65°C for 20 min to inactivate T4 DNA ligase. The sample was digested with 5 U of *Xba*I and 5 U of *Hha*I at 37°C for 15 min to cleave any restriction sites reformed by ligation.

Amplification. Each 50-μl PCR mixture included 0.5 μl of template DNA

* Corresponding author. Mailing address: Diagnostics and Molecular Epidemiology Section, Mailstop F08, Centers for Disease Control and Prevention, 1600 Clifton Rd., Atlanta, GA 30333. Phone: (404) 639-1280. Fax: (404) 639-1287.

TABLE 1. Related MAI isolates

Patient no.	Isolate no.	Sample used for culture	Culture date
1	98.1	Lymph node	June 1985
1	98.2	Bone marrow	June 1985
2	513.1	Sputum	April 1990
2	513.2	Sputum	March 1991
3	608.1	Blood	15 August 1990
3	608.2	Stool	13 August 1990
4	64.1	Sputum	20 April 1984
4	64.2	Sputum	20 April 1984
5	104.1	Bone marrow	1 August 1985
5	104.2	Stool	8 July 1986
5	104.3	Blood	June 1987
5	104.4	Blood	3 November 1987

(1/50th of the ligation-redigestion product), 0.25 U of *Taq* DNA polymerase (Boehringer Mannheim, Indianapolis, Ind.), deoxynucleoside triphosphates (200 μM each), and one or more oligonucleotide primers (1.0 μM) in 1× PCR buffer. Typically, the oligonucleotides AH1 and PX-G were used together as primers. The effect of deleting either of these primers or substituting oligonucleotides PX, PX-C, PX-A, or PX-T for PX-G was assessed in some experiments. Amplification was performed in a GeneAmp PCR System 9600 (Perkin-Elmer, Branchburg, N.J.) with an amplification profile that consisted of an initial denaturation step at 94°C for 5 min and then 30 cycles with denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and extension at 72°C for 90 s. The effect of varying the annealing temperature was assessed in some experiments. Three other thermal cyclers (GeneAmp PCR System 2400 [Perkin-Elmer], Coy Tempcycler II model 110S [Coy Laboratory Products Inc., Ann Arbor, Mich.], and DNA Thermal Cycler 480 [Perkin-Elmer]) were used to assess their effects on the outcome of PCR. The amplification profile was altered for the Coy Tempcycler II and the DNA Thermal Cycler 480. For these thermal cyclers the amplification profile consisted of an initial denaturation step at 94°C for 5 min and then 30 cycles with denaturation at 94°C for 60 s, primer annealing at 60°C for 60 s, and extension at 72°C for 120 s. All experiments included negative controls which were processed with the samples.

TABLE 2. Unrelated MAI, *P. aeruginosa*, and *S. aureus* isolates

Species	Isolate no.	Sample used for culture	City where organism was isolated
MAI	C1	Blood	Shreveport, La.
MAI	C2	Blood	Las Vegas, Nev.
MAI	C3	Blood	Wilmington, Maine
MAI	C4	Blood	Milwaukee, Wis.
MAI	C5	Blood	New York, N.Y.
MAI	C6	Blood	Albuquerque, N.M.
MAI	C7	Blood	Lexington, Ky.
MAI	C8	Blood	Minneapolis, Minn.
MAI	C9	Blood	Little Rock, Ark.
MAI	C10	Blood	Salt Lake City, Utah
MAI	C11	Blood	Oklahoma City, Okla.
MAI	C12	Blood	Wichita, Kans.
MAI	C13	Blood	Kansas City, Kans.
MAI	C14	Blood	Springfield, Mo.
MAI	C15	Blood	Portland, Oreg.
MAI	C16	Blood	Houston, Tex.
MAI	C17	Blood	Ann Arbor, Mich.
MAI	C18	Blood	Seattle, Wash.
MAI	C19	Blood	Jackson, Miss.
MAI	C20	Blood	Memphis, Tenn.
P. aeruginosa	ATCC 10145	Not specified	Not specified
P. aeruginosa	ATCC 17933	Sputum	Not specified
P. aeruginosa	ATCC 19143	Cutaneous wound	Not specified
P. aeruginosa	PA013	Sputum	Tyler, Tex.
S. aureus	SA101	Blood	Tyler, Tex.
S. aureus	SA105	Wound	Tyler, Tex.
S. aureus	SA109	Sputum	Tyler, Tex.
S. aureus	ATCC 29213	Wound	Not specified

TABLE 3. Oligonucleotides used in the study and their sequences

Designation	Sequence
AH1	5′-AGA ACT GAC CTC GAC TCG CAC G-3′[a]
AH2	5′-TGC GAG T-3′
AX1	5′-PO_4-CTA GTA CTG GCA GAC TCT-3′
AX2	5′-GCC AGT A-3′
PX	5′-AGA GTC TGC CAG TAC TAG A-3′
PX-G	5′-AGA GTC TGC CAG TAC TAG AG-3′
PX-C	5′-AGA GTC TGC CAG TAC TAG AC-3′
PX-T	5′-AGA GTC TGC CAG TAC TAG AT-3′
PX-A	5′-AGA GTC TGC CAG TAC TAG AA-3′

[a] This sequence is used for construction of the AH adaptor and as a primer in IRS-PCR.

Visualization of the electrophoretic pattern. The PCR products (18 μl) were loaded into wells (1.5 by 5 mm) of a 6.5% polyacrylamide gel prepared from a 30% acrylamide–bisacrylamide (29:1) solution (Bio-Rad Laboratories, Hercules, Calif.) in 1× TBE buffer (0.045 M Tris-borate, 0.001 M EDTA). After electrophoreses for 2 h at 200 V, the gel was stained with ethidium bromide (0.5 μg/ml) for 10 min, destained in water for 25 min, and photographed with UV illumination.

RESULTS

Strategy. The IRS-PCR scheme is illustrated in Fig. 1. The IRS-PCR template is produced by double digestion of genomic DNA with a restriction enzyme that cuts infrequently (*Xba*I; recognition sequence, T/CTAGA) and a restriction enzyme that cuts frequently (*Hha*I; recognition sequence, GCG/C) (Fig. 1A, panel 1). Double-stranded adaptors (AX and AH) are ligated to the cohesive ends of the restriction fragments (Fig. 1A, panel 2). Both adaptors consist of a long oligonucleotide and a short (seven-base) oligonucleotide. These oligonucleotides anneal at low temperature, allowing efficient ligation of the adaptor, but they dissociate at the higher temperatures. The short oligonucleotides do not participate in reactions subsequent to ligation. The longer oligonucleotide (AH1) in the *Hha*I adaptor (AH) is ligated to the recessed 5′ end of restriction fragments produced by *Hha*I digestion. The longer oligonucleotide (AX1) in the *Xba*I adaptor (AX) is phosphorylated to allow ligation to the recessed 3′ end of the restriction fragments. Oligonucleotide AH2 is not phosphorylated and, therefore, is not ligated. During PCR, the few fragments having the *Xba*I adaptor bind the primer PX (Fig. 1A, panel 4), and extension of this primer generates binding sites for primer AH1 (Fig. 1A, panel 5). This allows specific amplification of the desired *Xba*I-*Hha*I fragments during subsequent PCR cycles (Fig. 1A, panel 6). Fragments with *Hha*I sites at both ends are not amplified because there are no sites for annealing of AH1 (Fig. 1B).

Primer PX is complementary to the adaptor oligonucleotide AX2 plus the additional A that is present in the *Xba*I site. This primer will not amplify adaptor-dimers that may have formed during the ligation step. To increase the specificity of the procedure, four additional primers (PX-G, PX-C, PX-T, and PX-A) were synthesized, each having one additional 3′ base (G, C, T, or A, respectively). Each of these primers will amplify a subset of the *Xba*I-*Hha*I fragments, depending upon the next base adjacent to the *Xba*I site.

Patterns from MAI isolates. By using PX-G and AH1 as primers, IRS-PCR produced a unique pattern for each of 20 MAI isolates recovered in different cities (Fig. 2). Deletion of either primer from the reaction mixture, deletion of the re-

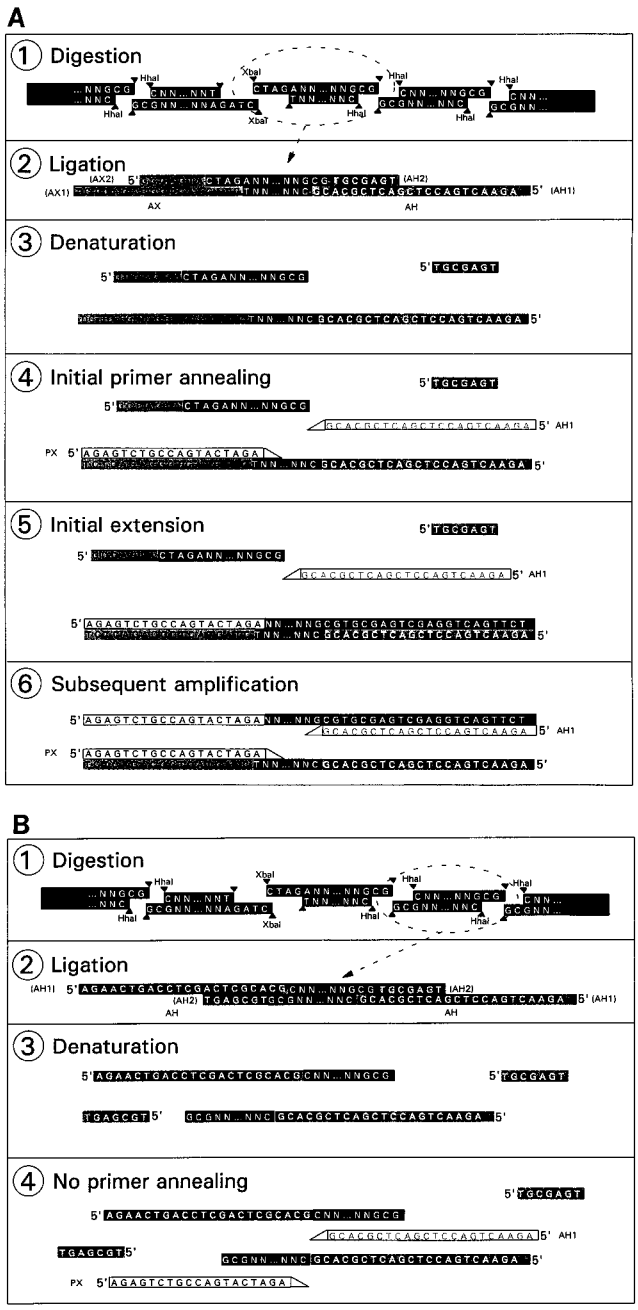

FIG. 1. IRS-PCR strategy. *Xba*I-*Hha*I restriction fragments are amplified (A). *Hha*I restriction fragments are not amplified (B).

strictive digestion step, or deletion of the ligation step prevented amplification. Unique patterns were also produced when PX-G was replaced with PX, PX-C, PX-A, or PX-T (data not shown). While the patterns generated with PX were characteristic of each strain, they contained a large number of bands which made comparisons difficult.

The patterns generated by IRS-PCR were reproducible. The patterns generated with multiple MAI isolates from the same patient were essentially identical but different from those generated with isolates recovered from different patients (Fig. 3). Twelve MAI isolates from five patients gave five patient-specific IRS-PCR patterns when PX-G and AH1 were used as

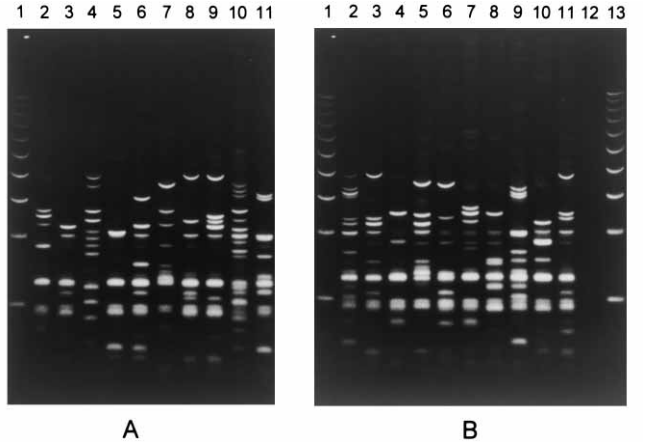

FIG. 2. IRS-PCR electrophoretic patterns of MAI isolates from patients in different geographic locations. (A) Lane 1, 100-bp ladder; lanes 2 through 11, MAI isolates recovered in Shreveport, La.; Las Vegas, Nev.; Wilmington, Maine; Milwaukee, Wis.; New York, N.Y.; Albuquerque, N.M.; Lexington, Ky.; Minneapolis, Minn.; Little Rock, Ark.; and Salt Lake City, Utah, respectively. (B) Lanes 1 and 13, 100-bp ladder; lanes 2 through 11, MAI isolates recovered in Oklahoma City, Okla.; Wichita, Kans.; Kansas City, Kans.; Springfield, Mo.; Portland, Oreg.; Houston, Tex.; Ann Arbor, Mich.; Seattle, Wash.; Jackson, Miss.; and Memphis, Tenn., respectively; lane 12, negative control. The IRS-PCR annealing temperature was 60°C, and the primers were PX-G and AH1.

primers. The same clustering of the MAI isolates was apparent following amplification with primer PX, PX-C, PX-A, or PX-T (data not shown).

Patterns from *P. aeruginosa* and *S. aureus* isolates. IRS-PCR with PX-G and AH1 as primers produced unique patterns for four *P. aeruginosa* isolates and four *S. aureus* isolates (Fig. 4). Deletion of either primer from the reaction mixture, deletion of the digestion step, or deletion of the ligation step prevented amplification. Unique patterns were also produced when PX-G was replaced with PX-C, PX-A, or PX-T (data not shown).

Thermal cycler and annealing temperature variations. No significant pattern differences were noted when the PCR an-

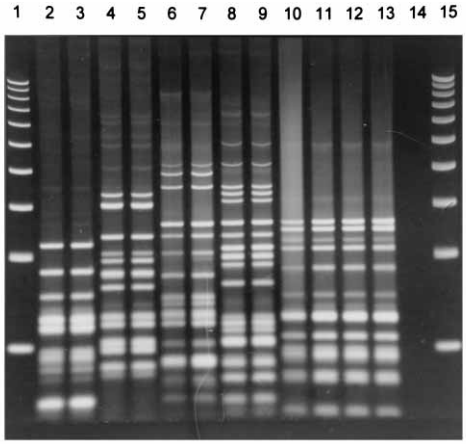

FIG. 3. IRS-PCR electrophoretic patterns of MAI isolates from multiple clinical specimens from five patients. Lanes 2 and 3, isolates 98.1 and 98.2, respectively, from patient 1; lanes 4 and 5, isolates 513.1 and 513.2, respectively, from patient 2; lanes 6 and 7, isolates 608.1 and 608.2, respectively, from patient 3; lanes 8 and 9, isolates 64.1 and 64.2, respectively, from patient 4; lanes 10 through 13, isolates 104.1, 104.2, 104.3, and 104.4, respectively, from patient 5; lane 14, negative control; lanes 1 and 15, 100-bp ladder. The IRS-PCR annealing temperature was 60°C, and the primers were PX-G and AH1.

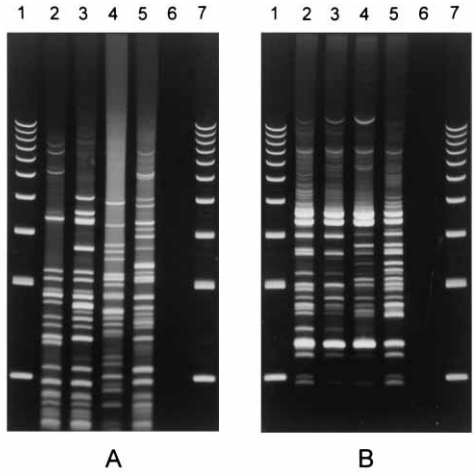

FIG. 4. IRS-PCR electrophoretic patterns of four *P. aeruginosa* isolates (A, lanes 2 through 5; isolates ATCC 10145, ATCC 17933, ATCC 19143, and PA013, respectively) and four *S. aureus* isolates (B; lanes 2 through 5, isolates SA101, SA105, SA109, and ATCC 29213, respectively). (A and B) lane 6, negative control; lanes 1 and 7, 100-bp ladder. The IRS-PCR annealing temperature was 60°C, and the primers were PX-G and AH1.

nealing temperature was varied from 45 to 65°C. (Fig. 5). No significant alterations were seen in the patterns for two MAI isolates when the primers PX-C and AH1 were used at the different annealing temperatures. The effect of altering the annealing temperature with other primer sets was not studied. No difference was detected when PCR was performed in different thermal cyclers with amplification profiles adjusted for each machine's format.

DISCUSSION

Identification of bacterial strains by DNA fingerprinting facilitates epidemiologic studies and disease control. We developed an approach for fingerprinting which is rapid, sensitive, and widely applicable. We used the approach to characterize acid-fast, gram-negative, and gram-positive organisms.

The key feature of the approach is the selective amplification of DNA sequences located between a frequently occurring endonuclease restriction site and an infrequently occurring restriction site. Adaptor and primer design dictate which restriction fragments are subsequently amplified. Amplification of the DNA flanking the infrequent restriction sites produces electrophoretic patterns specific enough to be referred to as DNA fingerprints. The design of our *Hha*I adaptor is similar to that of an adaptor described by Mueller and Wold (7) in that one oligonucleotide (AH2) is relatively short and the other is long. The short oligonucleotide allows efficient ligation of the double-stranded adaptor at 16°C but cannot form stable hybrids at higher temperatures and cannot compete for primer. An alternative method of eliminating the adaptor was described by Haas et al. (3). Those investigators incorporated a uracil-containing oligonucleotide into their double-stranded adaptor. Following ligation the uracil-containing oligonucleotide was eliminated by digestion with uracil-*N*-glycosidase and heat. Our design facilitates selective amplification with less manipulation, time, and expense.

The designs of our *Xba*I adaptor and the corresponding primers allow initiation of amplification and additional selectivity. Again, one strand of the adaptor (AX2) is short to prevent it from serving as primer during PCR. The use of primers that are longer than the oligonucleotides in the adaptor prevents the amplification of primer-dimers. Further increases in primer length (as done for primers PX-G, PX-C, PX-A, and PX-T) promotes an added degree of selectivity and facilitates the production of four sets of electrophoretic patterns. Thus, the relationships between isolates can be confirmed by examining the patterns produced with different primers. This confirmation can be reassuring when studying unknown isolates. In a similar fashion, primers to the *Hha*I adaptor could have been extended to produce subsets of amplified fragments and different electrophoretic patterns.

This method of strain identification can be completed in 6 to 8 h from the time of receipt of an isolate. Once the organism is isolated, there is no need for further growth; minute quantities of target DNA are sufficient for PCR amplification. Because the target DNA fragments are smaller than 1.5 kb, target DNA can be extracted from the organisms in a variety of ways, including mechanical lysis. While mechanical lysis is rapid, it causes considerable shearing of the genomic DNA. In comparison, isolation of high-molecular-weight DNA suitable for macrorestriction analysis is laborious and time-consuming. The fragments amplified by IRS-PCR are small, facilitating separation in 2 to 3 h by polyacrylamide gel electrophoresis. Detection of DNA polymorphisms by Southern blot hybridization requires specific probes; construction of these probes and hybridization are also time-consuming.

Other PCR-based assays of genetic diversity rely on arbitrary primers or require target DNA sequence information for the design of specific primers. The time and cost of obtaining the sequence information for many organisms have been prohibitive. A method described by Vos et al. (14) has some similarity to IRS-PCR in that the DNA is digested with two restriction enzymes and ligated to two adaptors; however, their method does not provide a mechanism for amplifying specific DNA fragments in the mixture. Small variations in temperature, target DNA concentration, or PCR buffer can significantly alter the pattern produced by arbitrary primers (15, 16). Other approaches for PCR amplification of DNA flanking known

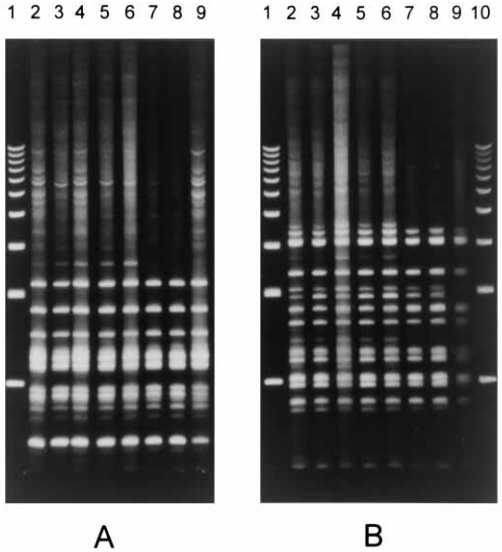

FIG. 5. Effects of altering the annealing temperature on the IRS-PCR electrophoretic patterns of two MAI isolates (MAI 98.2 [A] and MAI 513.1 [B]). Annealing temperatures were 45°C (lanes 2), 50°C (lanes 3), 52°C (lanes 4), 54°C (lanes 5), 56°C (lanes 6), 58°C (lanes 7), 60°C (lanes 8), and 65°C (lanes 9). Panel A, lane 1, and panel B, lanes 1 and 10, 100-bp ladder. The IRS-PCR primers were PX-G and AH1.

sequences (2, 3, 5, 7–10, 13) are not applicable to sequences as short as endonuclease recognition sites.

The IRS-PCR procedure can be performed with equipment that is already available in many clinical laboratories. This method of DNA fingerprinting is potentially applicable to a wide array of organisms. Identical enzymes, adaptors, primers, and PCR conditions were used to characterize 32 MAI isolates, 4 *P. aeruginosa* isolates, and 4 *S. aureus* isolates. The 32 MAI isolates from 25 patients gave 25 different, patient-specific IRS-PCR patterns with the primers PX-G and AH1. The four *P. aeruginosa* isolates and four *S. aureus* isolates gave patterns which were unique. Pulsed-field gel electrophoresis studies of these MAI, *P. aeruginosa*, and *S. aureus* isolates demonstrated identical clustering of the isolates (6; unpublished data).

Amplification of DNA from *Neisseria meningitidis* and *Staphylococcus epidermidis* isolates by using identical parameters suggests that IRS-PCR will be useful with these species as well (data not shown). No adaptations in the methods were required to fingerprint these organisms. However, if needed, the method is extremely adaptable. The number of fragments amplified and the complexity of the electrophoretic pattern can be adjusted by adjusting the length of either primer. The use of different enzymes can generate a set of different patterns. Like other restriction fragment length polymorphism methods, some enzymes may provide more or less discriminating patterns. Selection of an appropriate set of enzymes for comparing organisms can be based on known characteristics of their genome. A frequently cutting enzyme with a short recognition sequence rich in A and T residues may be more appropriate for organisms whose genomes are rich in these nucleotides. Lists of infrequently cutting enzymes are available for many organisms.

IRS-PCR fingerprinting is a potentially powerful epidemiologic tool. It is rapid, widely applicable, reproducible, and highly adaptable. We hope that it facilitates epidemiologic studies and improves disease control.

ACKNOWLEDGMENTS

We thank Vickie Baselski, Joe E. Humphries, Elaine V. Condon, Dheryl McMillan, Neva Bus, Sharon Poor, Drieda Booth, E. H. Gerlach, Mary S. Lewis, Dewey Hansen, Grace Onyi, Walter Pace, John Besser-Wiek, Mary Beth Cook, Philip M. Tierno, Dona Eury, Julie Patterson, Arnold D. Oberle, David Murphy, and Ali Azghani for providing bacterial isolates. We thank Bonnie Plikaytis and Shanti Kunchaparty for advice on optimizing PCR.

This study was partially supported by a grant from the Lizanell and Colbert Coldwell Foundation.

REFERENCES

1. **Bhaduri, S., and P. H. Demchick.** 1983. Simple and rapid method for disruption of bacteria for protein studies. Appl. Environ. Microbiol. **46:**941–943.
2. **Frohman, M. A., M. K. Dush, and G. R. Martin.** 1988. Rapid production of full length cDNA's from rare transcripts using single side gene specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA **85:**8998–9002.
3. **Haas, W. H., W. R. Butler, C. L. Woodley, and J. T. Crawford.** 1993. Mixed-linker polymerase chain reaction: a new method for rapid fingerprinting of isolates of the *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **31:** 1293–1298.
4. **Levy-Frebault, V. V., M. F. Thorel, A. Varnerot, and B. Gicquel.** 1989. DNA polymorphism in *Mycobacterium paratuberculosis*, "wood pigeon mycobacteria," and related mycobacteria analyzed by field-inversion gel electrophoresis. J. Clin. Microbiol. **27:**2823–2826.
5. **Loh, E. Y., J. F. Elliot, S. Cweria, L. L. Lanier, and M. M. Davis.** 1989. Polymerase chain reaction with single sided specificity: analysis of T-cell receptor delta chain. Science **243:**217–220.
6. **Mazurek, G. H., S. Hartman, Y. Zhang, B. A. Brown, J. S. R. Hector, D. Murphy, and R. J. Wallace, Jr.** 1993. Large DNA restriction fragment polymorphism in the *Mycobacterium avium-M. intracellulare* complex: a potential epidemiologic tool. J. Clin. Microbiol. **31:**390–394.
7. **Mueller, P. R., and B. Wold.** 1989. In vivo footprinting of a muscle specific enhancer by ligation mediated PCR. Science **246:**780–786.
8. **Ochman, H., A. S. Gerber, and D. L. Harti.** 1988. Genetic applications of an inverse polymerase chain reaction. Genetics **120:**621–623.
9. **Peiffer, G. P., S. D. Steigerwald, P. R. Mueller, B. Wold, and A. D. Riggs.** 1989. Genomic sequencing and methylation analysis by ligation mediated PCR. Science **246:**810–813.
10. **Riley, J., R. Butler, D. Oglivie, R. Finniear, D. Jenner, S. Powell, R. Anand, J. C. Smith, and A. F. Markham.** 1990. A novel, rapid method for the isolation of terminal sequences from yeast artificial chromosome (YAC) clones. Nucleic Acids Res. **18:**2887–2890.
11. **Smith, C. L., and C. R. Cantor.** 1987. Purification, specific fragmentation, and separation of large DNA molecules. Methods Enzymol. **155:**449–467.
12. **Tenover, F. C.** 1988. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev. **1:**82–101.
13. **Triglia, T., M. G. Peterson, and D. J. Kemp.** 1988. A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequence. Nucleic Acids Res. **16:**8186.
14. **Vos, P., R. H. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau.** 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. **23:**4407–4414.
15. **Welsh, J., and M. McClelland.** 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **18:**7213–7218.
16. **Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Pafalski, and S. V. Tingey.** 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18:**6531–6535.