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# Rapid Discrimination of *Mycobacterium avium* Strains from AIDS Patients by Randomly Amplified Polymorphic DNA Analysis

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**A randomly amplified polymorphic DNA (RAPD) analysis was performed for the molecular typing of *Mycobacterium avium* strains. This method was applied to epidemiologically unrelated *M. avium* strains isolated from the blood of 10 different AIDS patients and to strains that were considered epidemiologically related, as they had been isolated from the same patient but from different body locations (4 patients, 10 strains). Three oligonucleotide primers among the six tested were found to generate RAPD profiles with DNA from all *M. avium* strains and to successfully type them. This method for the typing of *M. avium* strains is rapid and easy to perform.**

Disseminated infections with organisms of the *Mycobacterium avium* complex (MAC) develop in as many as 40% of patients with advanced AIDS in developed countries (8). MAC comprises environmental mycobacteria; they have been isolated from various sources, including air, dust, soil, and water, but the specific source of MAC causing human infection has not been identified. However, it has recently been reported that persistent colonization of potable water can be a source of MAC infection in AIDS patients (19). Investigations of the sources of MAC infection as well as of the routes of transmission require the use of suitable epidemiological markers. Different laboratory methods have been applied to these purposes (4). Serotyping has been used extensively (18), but because of the predominance of a limited number of serotypes, this method is of limited use for *M. avium* epidemics. Multilocus enzyme electrophoresis (20), restriction fragment length polymorphism, and hybridization to specific probes (3), as well as restriction fragment profiles resolved by pulsed-field electrophoresis (7), appear to be suitable epidemiological tools for studying MAC infection. A less complex, PCR-based typing method was first described by Ross and Dwyer (12) and used more recently by Picardeau and Vincent (10) for typing *M. avium* isolates. These two groups have used primers that bound to the ends of insertion elements, thus amplifying DNA between closely spaced copies of these elements.

Randomly amplified polymorphic DNA (RAPD) analysis is a DNA fingerprinting method which has been used successfully to type different microbes. This method does not require knowledge of the genetic structure of the target, since there always will exist low-stringency priming sites for a single primer on both strands of the DNA at positions close enough to permit PCR amplification. Recently, the use of RAPD analysis for the typing of *Mycobacterium tuberculosis* strains has been reported (5).

The aim of the present study was the evaluation of RAPD analysis for the discrimination of MAC isolates. Ten *M. avium* strains isolated from the blood of different AIDS patients were studied comparatively. *M. avium* strains isolated from the same AIDS patients but from different body locations (four patients, 10 strains) were also included in the study. These *M. avium* strains had been isolated from the sputum, blood, and bone marrow (two of four patients) or from the sputum and blood (the two remaining patients).

*M. avium* growth was obtained on Lowenstein-Jensen slants. Mycobacterial DNA was extracted from distinct colonies with a DNA extraction reagent containing the Chelex anion-exchange resin (Perkin-Elmer, Branchburg, N.J.) as follows. Bacteria were removed from the Lowenstein-Jensen slants and suspended in distilled water. The Chelex reagent was added to the suspensions, and after boiling for 25 min and centrifugation, the supernatants were used for RAPD analysis. DNA extraction for some strains was performed in parallel with the Isoquick extraction kit (ORCA Research Inc., Bothell, Wash.), by following the instructions provided by the manufacturer. All isolates used in this study were identified as being *M. avium* by using specific probes (Gen-Probe, Chatillon, France) and a PCR method with MT6 and MT7 primers specific for *M. avium* (17). The results obtained by this PCR method with the specific primers were also used as positive controls for the PCR process and for the extraction process, while results from the amplification of distilled water in the place of *M. avium* DNA were used as the negative control for the PCR process. Six primers were used for the RAPD analysis (Table 1). The AV6/AV7 and A1245/B1245 sets of primers have already been used for the specific amplification of MAC strains by classical PCR (2, 17). The IR (inverse repeat) primer corresponds to the inverse repeat sequence of the insertion sequence IS*6110* of the *M. tuberculosis* complex (16). The Leg1 primer (in association with the Leg2 primer) is used by us and others (15) for the specific amplification of *Legionella pneumophila* strains.

PCR amplifications were performed in a total volume of 50 μl. The PCR mixture consisted of 50 mM Tris-HCl (pH 8.5), 17 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 6.7 μM EDTA, 10 mM β-mercaptoethanol, 0.1 mg of bovine serum albumin per ml, 0.01% gelatin, 200 μmol of each deoxynucleotide triphosphate, and 0.5 U of *Taq* DNA polymerase per reaction (Perkin-Elmer Cetus). Ten microliters of the DNA eluted from the Chelex resin (or 5 μl with the Isoquick kit) was usually added. The arbitrary primers were used in an amount of 100 pmol. The

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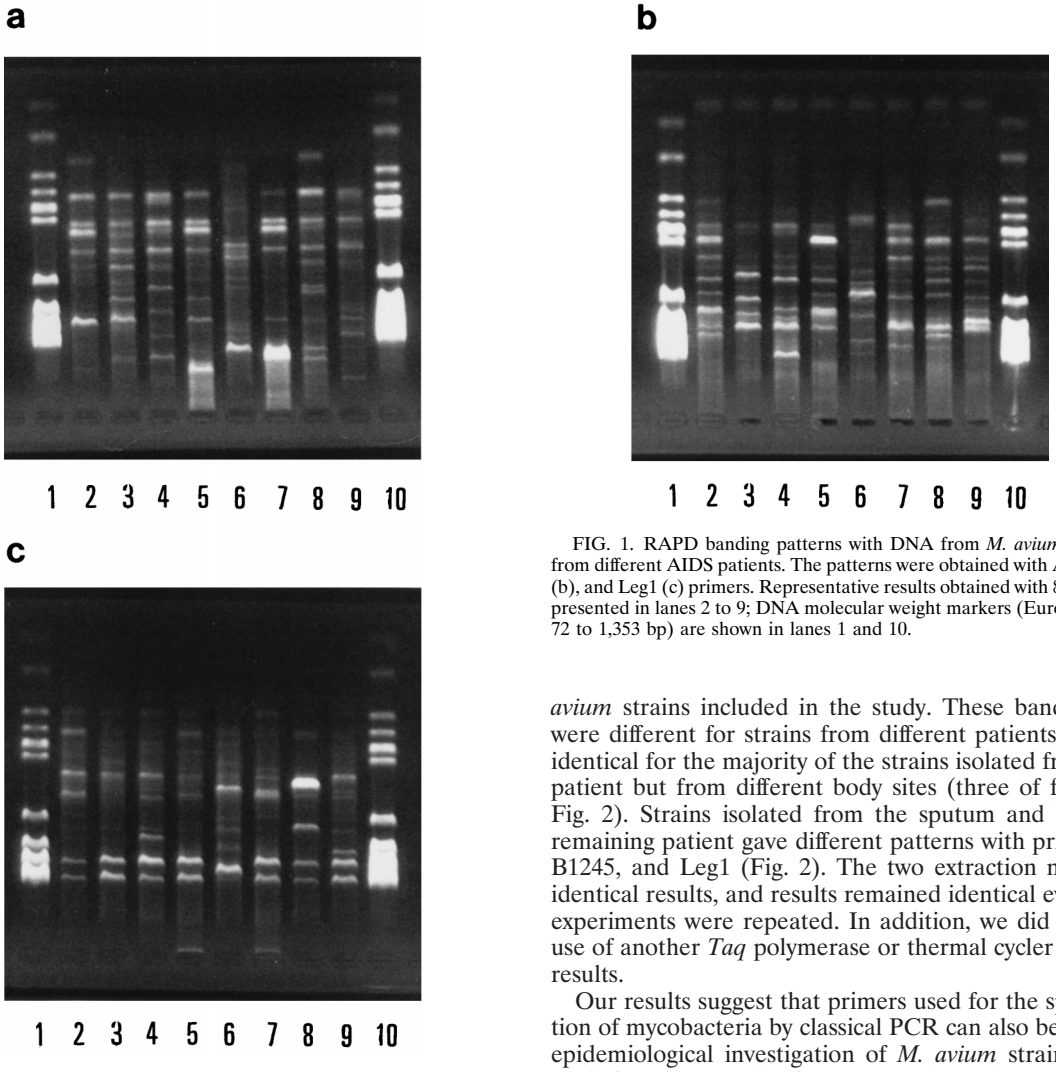


FIG. 1. RAPD banding patterns with DNA from *M. avium* strains isolated from different AIDS patients. The patterns were obtained with A1245 (a), B1245 (b), and Leg1 (c) primers. Representative results obtained with 8 of 10 strains are presented in lanes 2 to 9; DNA molecular weight markers (Eurogentec IX; from 72 to 1,353 bp) are shown in lanes 1 and 10.

reaction mixtures were overlaid with 100 μl of paraffin oil and incubated for 5 min at 94°C. A total of 36 cycles (92°C for 1 min, 35°C for 1 min, 72°C for 2 min) were performed in a Hybaid thermal cycler (Hybaid Limited, Middlesex, United Kingdom). After the final cycle, there was an elongation step of 7 min at 72°C. DNA fragments were then separated in a 2% agarose gel by electrophoresis and visualized by ethidium bromide staining. Gels were photographed, and band patterns were compared visually. Band staining intensity was not considered as a discriminatory factor, and profiles were considered unrelated if they were different in more than one band. To assess the reproducibility of the method, all RAPD experiments were performed twice. The RAPD analysis was also performed with another *Taq* polymerase (0.5 U/reaction; Pharmacia Biotech, St. Quentin en Yvelines, France) and thermal cycler (Crocodile II; Appligene, France).

The RAPD analysis performed with DNA from *M. avium* strains, and the AV6 primer was found to generate no banding patterns. Primers AV7 and IR were found to generate RAPD patterns with some but not all of the *M. avium* strains. This was still the case when the two primers were used together or when the *Taq* polymerase and the thermal cycler were changed.

The remaining three primers (A1245, B1245, and Leg1) generated RAPD banding patterns with DNA from all *M. avium* strains included in the study. These banding patterns were different for strains from different patients (Fig. 1) and identical for the majority of the strains isolated from the same patient but from different body sites (three of four patients; Fig. 2). Strains isolated from the sputum and blood of the remaining patient gave different patterns with primers A1245, B1245, and Leg1 (Fig. 2). The two extraction methods gave identical results, and results remained identical even when the experiments were repeated. In addition, we did not find that use of another *Taq* polymerase or thermal cycler modified the results.

Our results suggest that primers used for the specific detection of mycobacteria by classical PCR can also be used for the epidemiological investigation of *M. avium* strains by RAPD analysis. The set of primers A1245/B1245 amplify insertion element IS*1245*, which is specific for *M. avium* strains, in high-stringency conditions, and their PCR product can be used as a probe in RFLP analysis (2). The possibility that, in our experiments, these primers specifically amplified only interinsertion sequence regions cannot be excluded, but this seems unlikely given the low annealing temperature, which encourages nonspecific hybridization of primers. The hypothesis we favor is that the characteristics of the primers that specifically amplify mycobacterial DNA by classical PCR correspond also to the characteristics necessary for nonstringent interactions, such as

TABLE 1. Six primers tested for generation of RAPD profiles with DNA from *M. avium* strains

| Primer | Sequence (5′ to 3′) | RAPD profile[a] |
|---|---|---|
| AV6 | ATGGCCGGGAGACGATCTATGCCGGCGTAC | NB |
| AV7 | CGTTCGATCGCTGTTTGTGCAGCGCGTACA | B-SS |
| IR | CCCGGCATGTCCGGAGACTC | B-SS |
| A1245 | GCCGCCGAAACGATCTAC | GD |
| B1245 | AGGTGGCGTCGAGGAAGAC | GD |
| Leg1 | GTCATGAGGAATCTCGCTG | GD |

[a] NB, no bands; B-SS, some strains give bands; GD, bands produced, good discrimination of strains.

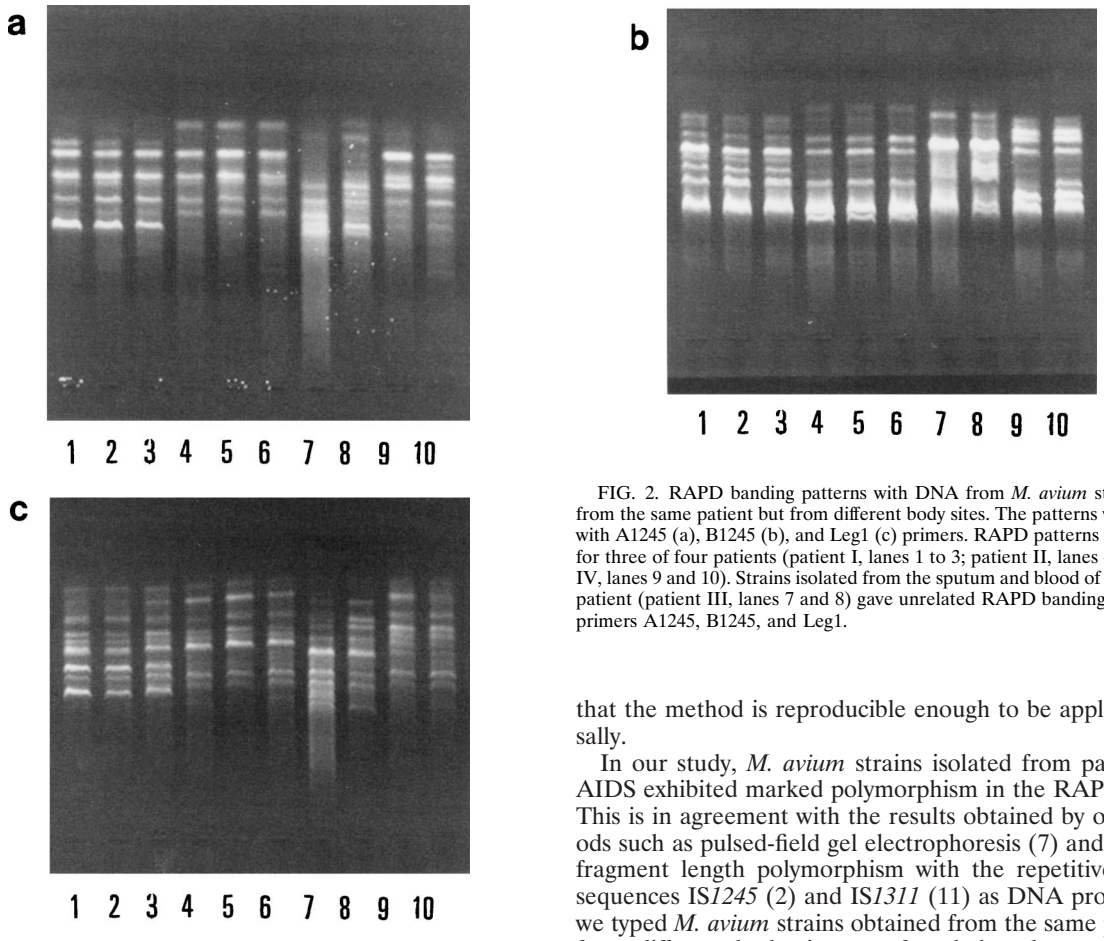


FIG. 2. RAPD banding patterns with DNA from *M. avium* strains isolated from the same patient but from different body sites. The patterns were obtained with A1245 (a), B1245 (b), and Leg1 (c) primers. RAPD patterns were identical for three of four patients (patient I, lanes 1 to 3; patient II, lanes 4 to 6; patient IV, lanes 9 and 10). Strains isolated from the sputum and blood of the remaining patient (patient III, lanes 7 and 8) gave unrelated RAPD banding patterns with primers A1245, B1245, and Leg1.

GC content relatedness. We have previously reported that the inverse repeat sequence derived from the IS*6110* element, which is specific for the *M. tuberculosis* complex, can also be used for *L. pneumophila* typing by RAPD analysis (6). Conversely, the Leg1 primer, which in this study gave satisfactory results for *M. avium* typing, is specific for *L. pneumophila* and is not known to specifically amplify the mycobacterial genome. The fact that both mycobacteria and *Legionella* spp. have high GC content could explain why primers that specifically amplify mycobacteria can also be used for the typing of *Legionella* and vice-versa.

The results described by Linton et al. (5) are in agreement with this hypothesis. They found that the three most satisfactory primers, among 40 tested, for the RAPD analysis of *M. tuberculosis* strains were the ones originally designed to amplify regions of the *M. tuberculosis* genome by classical PCR. The original targets in this study were also insertion sequences, but the intensities of the amplicon bands decreased rapidly as the annealing temperature was increased above 40°C. The specific amplification of interinsertion sequence regions was therefore considered unlikely.

PCR-based techniques remain highly attractive, since they are easy to perform, but RAPD analysis is known to present problems in obtaining reproducible results. Diverse factors, such as the type of thermal cycler (9) and *Taq* polymerase (13) used, have been proposed as affecting the results obtained. In our study, we did not find that these two factors interfered with our results, but further studies are required before we can state that the method is reproducible enough to be applied universally.

In our study, *M. avium* strains isolated from patients with AIDS exhibited marked polymorphism in the RAPD analysis. This is in agreement with the results obtained by other methods such as pulsed-field gel electrophoresis (7) and restriction fragment length polymorphism with the repetitive insertion sequences IS*1245* (2) and IS*1311* (11) as DNA probes. When we typed *M. avium* strains obtained from the same patient but from different body sites, we found that these strains were identical for three of four patients. The strains isolated from the blood and sputum of the remaining patient were found to be different. Other groups using pulsed-field gel electrophoresis methods have previously reported that AIDS patients can present polyclonal MAC infections (1, 14). It is therefore clear that the RAPD analysis can be used to type unrelated *M. avium* strains and to detect polyclonal *M. avium* infections in patients with AIDS.

In conclusion, this study shows that the RAPD analysis is appropriate and useful for the typing of *M. avium* strains. Its simplicity and speed should render it applicable for large epidemiological studies.

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