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Isolation of Specific DNA Fragments of *Mycobacterium avium* and Their Possible Use in Diagnosis

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We cloned and sequenced two DNA fragments (DT1 and DT6) from *Mycobacterium avium* serotype 2 for use in the identification of members of the *M. avium-M. intracellulare* complex (MAC). Reference strains of MAC belonging to serovars 1 to 28 were examined by using these DNA fragments as probes. The study revealed that the DT6 probe hybridized with DNAs from *M. avium* strains (serovars 1 to 6, 8 to 11, and 21), while the DT1 probe hybridized with DNAs from serovars 2, 3, 7, 12 to 20, and 23 to 25. DT1- and DT6-derived oligonucleotides were selected for use as primers in a polymerase chain reaction test. Amplification of the DT1 and DT6 sequences may provide the basis for a rapid and reliable assay for the detection of mycobacteria belonging to MAC.

Mycobacterium avium is a potential pathogen involved in a large variety of diseases in humans and animals, and its role as the agent of newly recognized mycobacterial diseases has recently been reviewed (24). In non-AIDS patients, *M. avium* mainly causes pulmonary infections in adults and cervical adenopathies in children, whereas digestive or disseminated infections are highly frequent in patients with AIDS. In patients with AIDS, *M. tuberculosis* and *M. avium* represent up to 90% of the agents involved in mycobacterial diseases. Furthermore, *M. avium* is an important pathogen for animals, causing tuberculosis in birds and lymph node infections in cattle and swine.

DNA-DNA hybridization studies demonstrated that *M. paratuberculosis*, the agent of an enteric disease in cattle seldom isolated from patients with Crohn's disease as well as another animal pathogen, designated wood pigeon bacillus, which is able to produce paratuberculosis in cattle and tuberculosis in birds, belonged to the same genomic species *M. avium* (13, 18, 25). The nomenclature has accordingly been revised and the subspecies *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* (standing for the trivial designation "wood pigeon bacillus") have been introduced (20). Recognition of the subspecies within *M. avium* species is determined by phenotypic features, especially mycobactin dependence and ability to grow on egg medium (20).

However, phenotypic differentiation of *M. avium* from *M. intracellulare*, a distinct genomic species, is not performed by usual identification tests but can be achieved by serotype determination. According to the first classification based on serotyping and developed by Schaefer (19) and Good and Beam (6), serotypes 1 to 3 were considered *M. avium*, whereas the other serotypes corresponded to *M. intracellulare*. Twenty-eight serotypes within the *M. avium-M. intracellulare* complex have been described. In a pioneering DNA-DNA hybridization study, Baess (1) demonstrated that serotypes 1 to 6 and 8 belong to *M. avium*, whereas serotypes 7, 12, 14, 16, and 18 belong to *M. intracellulare*. Classification of the other serotypes has not been performed by this reference classification method.

DNA probes for the identification of *M. avium* and *M. intracellulare* (5) are commercially available (Gen-Probe, San Diego, Calif.) and have been evaluated in several studies (7, 11, 12, 14).

Because of the high frequency of *M. avium* isolation in human clinical samples, the increasing importance of *M. avium* in pathology and its prominent role in patients with AIDS on the one hand and the difficulty of identification of the species on the other hand, we undertook a search for DNA sequences specific for *M. avium* and *M. intracellulare* to provide probes for their rapid and reliable identification.

MATERIALS AND METHODS

Mycobacterial strains. The mycobacterial species type strains used in the present work are listed in Table 1. In addition, 28 reference serotypes of the *M. avium* complex, kindly provided by A. Tsang, were used (21, 22). The *Tsukamurella paurometabelum* type strain was also included in the study.

Construction of an *M. avium* cosmid library. *M. avium* serovar 2 (ATCC 25291) genomic DNA was partially digested for 1 h at 37°C with 0.03 U of *SalI* per μg of total DNA. DNA fragments were fractionated by electrophoresis on a 0.6% agarose gel in TAE (0.04 M Tris acetate, 0.001 M EDTA). The 30- to 40-kb fragments were electroeluted in TBE (0.089 M Tris borate, 0.089 M boric acid, 0.002 M EDTA) in dialysis membranes (17). Fragments (1.5 μg) were ligated with 700 ng of the pHC79 cosmid (9) that was previously digested with *SalI* and dephosphorylated with alkaline phosphatase. Ligation was done at 14°C for 16 h with 2.5 U of T4 DNA ligase in the buffer supplied by the manufacturer. Recombinant cosmids were packaged (10) and used to infect *Escherichia coli* HB101. Ampicillin-resistant and tetracycline-susceptible colonies were picked and grown in ampicillin-supplemented LB broth. Cosmid DNAs were extracted by the rapid alkaline extraction procedure.

Isolation of specific fragments from the *M. avium* genomic library. Cosmid DNAs were digested to completion with

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TABLE 1. Sources of genomic DNAs used for Southern analysis and/or PCR

Strain	Origin[a]
M. asiaticum	ATCC 25276
M. avium	ATCC 25291
M. avium subsp. *paratuberculosis*	ATCC 19698
M. bovis	ATCC 19210
M. bovis BCG Pasteur	CIPT 14 004 0001
M. chelonae	NCTC 946
M. fallax	CIPT 14 139 0005
M. flavescens	ATCC 14474
M. fortuitum	ATCC 6841
M. gastri	ATCC 15754
M. gordonae	ATCC 14470
M. intracellulare	ATCC 13950
M. kansasii	ATCC 12478
M. malmoense	ATCC 29571
M. microti	NCTC 8710
M. nonchromogenicum	ATCC 19530
M. scrofulaceum	ATCC 19981
M. shimoidei	ATCC 27962
M. simiae	ATCC 25275
M. smegmatis	ATCC 19420
M. szulgai	NCTC 10831
M. terrae	ATCC 15755
M. thermoresistibile	ATCC 19527
M. triviale	ATCC 23292
M. tuberculosis	ATCC 27294 H37Rv
M. xenopi	NCTC 10042
T. paurometabolum	CIPT 14 200 0013

[a] ATCC, American Type Culture Collection, Rockville, Md.; CIPT, Collection Institut Pasteur Tuberculose, Paris, France; NCTC, National Collection of Type Cultures, London, United Kingdom.

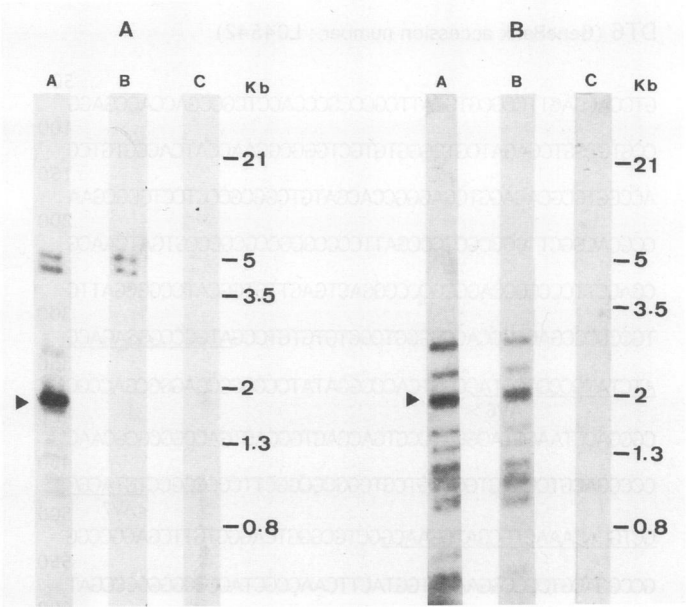

FIG. 1. Southern analysis of cosmids I1 and I6. A total of 15 μl of DNA was digested with the restriction enzyme *Sal*I. After electrophoresis, DNAs were transferred onto filters by Southern blotting and were hybridized with radiolabeled probes: *M. avium* serovar 2 (lane A), *M. intracellulare* serovar 16 (lane B), and *M. tuberculosis* (lane C) total DNA. The arrowheads indicate the 1.5-kb *M. avium*-specific fragment (A) and the 2-kb *M. avium-M. intracellulare*-specific fragment (B).

*Sal*I and electrophoresed through 0.6% agarose gels. Gels were incubated for 15 min in 0.25 M HCl, twice for 15 min each time in 1.5 M NaCl containing 0.5 M NaOH, and twice for 15 min each time in 1 M CH_3COONH_4 and were transferred onto Hybond N filters (Amersham). Filters were successively probed with [α-^{32}P]dCTP-labeled genomic DNA from *M. avium* serovar 2 (ATCC 25291), *M. intracellulare* serovar 16 (ATCC 13950), and *M. tuberculosis* H37Rv (ATCC 27294).

A *Sal*I fragment (named I1) which specifically hybridized with DNAs from *M. avium* serotype 2 and *M. intracellulare* serotype 16 and a *Sal*I fragment (named I6) which hybridized only with *M. avium* DNA were electroeluted from the agarose gels and were sequenced by the chain termination method (17) by using *Taq* polymerase and 7-deaza-dGTP instead of dGTP to prevent band compression in the GC-rich regions.

Preparation of the DNA probes. From the I1 and I6 fragments described above, a 876-bp *Sal*I-*Bam*HI fragment (DT1) and a 719-bp *Sal*I-*Eco*RI fragment (DT6), respectively, were subcloned in pUC18. The resulting plasmids were named pMA01 and pMA02, respectively.

The DT1 and DT6 DNA fragments were purified from the plasmid vector by restriction enzyme digestion, agarose gel electrophoresis, and electroelution of the insert DNA from the gel slices. Fragments were radiolabeled with [α-^{32}P] dCTP by using the multiprime random-labeling method and were used as probes in Southern blot analysis.

Preparation of genomic DNAs. Strains were grown for 4 weeks at 37°C in 100 ml of 7H9 Middlebrook medium supplemented with ADC (Difco). The cells were then centrifuged for 30 min at 7,000 × *g*, resuspended in 7H9 medium supplemented with D-cycloserine (100 μg/ml)–glycine (14 μg/ml)–EDTA (6 mM)–lysozyme (200 μg/ml), and were allowed to grow for 48 h at 37°C. Cells were then centrifuged and were incubated for 18 h at 37°C in 4 ml of lysis solution (0.05 M Tris, 0.05 M EDTA, 0.1 M NaCl [pH 8], 0.5% sodium dodecyl sulfate [SDS], pronase [60 μg/ml]). DNA was then extracted with phenol-chloroform and ethanol precipitated at −20°C for 18 h. DNA was centrifuged, washed with 70% ethanol, and dissolved in TE buffer (0.01 M Tris-HCl [pH 8.0], 0.001 M EDTA).

Southern hybridization analysis of mycobacterial genomic DNA. Total DNA (1 μg) from MAC serovars 1 to 28, *M. tuberculosis* complex (*M. tuberculosis*, *M. bovis* BCG, and *M. bovis*), and other mycobacteria (*M. avium* subsp. *paratuberculosis*, *M. scrofulaceum*) were digested with 70 U of restriction endonuclease *Pst*I in the appropriate buffer for 16 h at 37°C. DNA fragments were separated by an overnight electrophoresis on a 0.6% agarose gel. The DNAs were then transferred onto a Hybond N filter (Amersham) by the Southern method (17) and were hybridized with denatured ^{32}P-radiolabeled DT1 or DT6 fragments.

Synthetic oligonucleotides. Oligonucleotides were synthesized on a Cyclone plus DNA synthesizer (Millipore-Waters) by the phosphoramidite coupling method. For generation and subsequent purification, we followed the manufacturer's instructions.

Polymerase chain reaction (PCR) technique. Amplification reactions were performed in 50-μl volume reactions containing 50 mM Tris-HCl (pH 8.5), 2 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 100 pmol of each primer, 200 μM (each) the four deoxyribonucleoside triphosphates (dATP, dGTP, dTTP, and dCTP), 1 ng of template DNA (in 5 μl), and 2 U of *Thermus aquaticus* DNA polymerase (Perkin-

FIG. 2. Sequences of the *SalI-Eco*RI fragment (DT6) from the I6 clone and the *SalI-Bam*HI fragment (DT1) from the I1 clone used for the hybridization and PCR experiments. The sequences used for priming the PCR (probes AV6 and AV7 and probes IN38 and IN41) are underlined. AV6 and AV7 directed the amplification of a 187-bp fragment, whereas IN38 and IN41 directed the amplification of a 666-bp fragment.

Elmer Corp., Norwalk, Conn.). The amplification mixture was overlaid with 50 μl of mineral oil and was subjected to 25 cycles of amplification as follows. Samples were incubated at 94°C for 1 min to denature the DNA, 60°C for 1 min to anneal the primers, and 72°C for 1 min to extend the annealed primers. Thermal cycling was performed in a programmable heat block (Gene ATAQ controller; Pharmacia). Each amplification experiment included a negative control sample without DNA and a positive control sample with 1 ng of *M. avium* serotype 2 (ATCC 25291) DNA.

In order to check the specificity of the amplified fragment, 10% of the amplification reaction was analyzed by electrophoresis on a 2% agarose gel by using ϕX174 DNA hydrolyzed with *Hin*cII (Pharmacia) as a marker. Gels were stained with ethidium bromide, photographed on a UV transilluminator, and analyzed by the Southern blot technique by using the ^{32}P-labeled DT1 and DT6 fragments as probes.

Hybridization experiments. Hybridizations were performed at 68°C for 16 h (17) in a mixture comprising 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate buffer [pH 7]), 5× Denhardt's solution, 0.5% SDS, 100 μg of denatured salmon sperm DNA per ml, and the DNA probe (10^6 cpm/ml). After hybridization, filters were washed twice with 2× SSC at 65°C for 10 min, once with 2× SSC–0.1% SDS at 65°C for 30 min, and once with 0.1× SSC at 65°C for 10 min. Filters were briefly air dried and exposed to Kodak XAR5 film with intensifying screens at −80°C.

Nucleotide sequence accession numbers. The DNA sequences of DT1 and DT6 have been deposited in GenBank and have the accession numbers L04543 and L04542, respectively.

RESULTS

Isolation of the probes specific for the *M. avium-M. intracellulare* complex. With the aim of finding *M. avium-M. intracellulare*-specific DNA sequences, we constructed a cosmid library representative of the complete *M. avium* serotype 2 genome. A set of 150 recombinant clones was analyzed by Southern blotting by using *M. avium* serotype 2, *M. intracellulare* serotype 16, and *M. tuberculosis* total DNA as probes after *Sal*I digestion. Several clones were found to contain fragments that hybridized strongly with *M. avium* and/or *M. intracellulare* DNA but not with *M. tuberculosis* DNA. Typical results for clones I1 and I6 are shown in Fig. 1. Clone I6 contained a 1.5-kb fragment that gave a strong signal with *M. avium*, while clone I1 contained a 2-kb fragment that hybridized with *M. avium* and *M. intracellulare* DNAs. None of the two fragments hybridized with *M.*

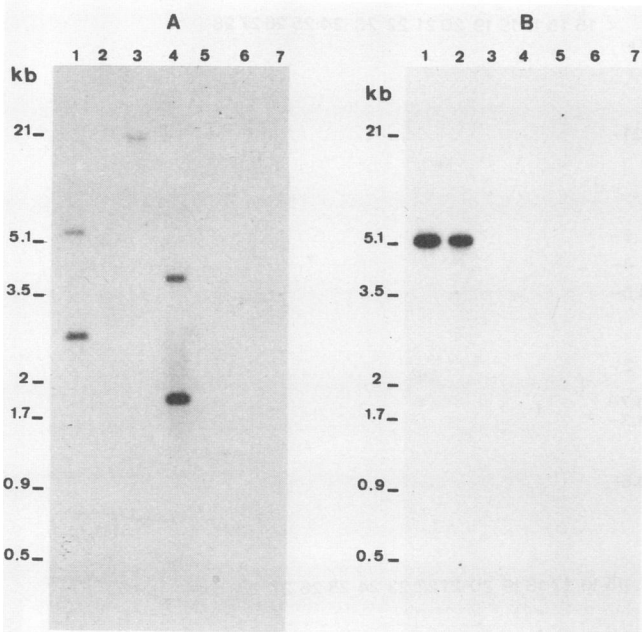

FIG. 3. Southern analysis of total mycobacterial DNAs probed with a 719-bp *Sal*I-*Eco*RI fragment (DT6) from I6 and an 876-bp *Sal*I-*Bam*HI fragment (DT1) from I1. A total of 1 μg of genomic DNAs from *M. avium* serovar 2 (lanes 1), *M. intracellulare* serovar 16 (lanes 2), *M. scrofulaceum* (lanes 3), *M. paratuberculosis* (lanes 4), *M. tuberculosis* (lanes 5), *M. bovis* BCG (lanes 6), and *M. bovis* (lanes 7) was digested to completion with restriction enzyme *Pst*I. After electrophoresis, DNAs were transferred onto a filter by Southern blotting and were hybridized with the radiolabeled DT6 (A) and DT1 (B) fragment.

tuberculosis DNA. These two fragments were purified from an agarose gel and cloned in plasmid pUC 18, and their nucleotide sequences were determined. A search of the EMBL and GenBank data bases did not reveal significant homologies with previously described sequences. A 876-bp *Sal*I-*Bam*HI fragment (DT1) and a 719-bp *Sal*I-*Eco*RI fragment (DT6) were isolated from the I1 and I6 sequences, respectively, and were subcloned in pUC18. The inserts that were retrieved were used as DNA probes for subsequent hybridization experiments. The complete sequences of the DT1 and DT6 fragments are reported in Fig. 2.

Specificities of the DT1 and DT6 probes. The specificities of the DT1 and DT6 probes were tested by using several mycobacterial type strains. The genomic DNAs were extracted from *M. avium*, *M. intracellulare*, *M. avium* subsp. *paratuberculosis*, *M. scrofulaceum*, *M. tuberculosis*, *M. bovis*, and *M. bovis* BCG. These DNAs were completely digested with *Pst*I and were probed with the DT1 and DT6 fragments by Southern blotting analysis. The results are shown in Fig. 3. By using DT1 as the probe, hybridization was observed only with *M. avium* and *M. intracellulare* DNAs. While using DT6 as the probe, a strong hybridization signal was observed with *M. avium* and *M. avium* subsp. *paratuberculosis* and a weak hybridization signal was obtained with *M. scrofulaceum*. DT6 did not hybridize with DNA from *M. intracellulare* or the *M. tuberculosis* complex.

Then, the DT1 and DT6 probes were used in Southern hybridization analysis of the 28 serovars belonging to the *M. avium-M. intracellulare* complex and 2 serovars (41 and 42) belonging to the *M. scrofulaceum* complex. *Pst*I was used for the DNA digestion. When DT6 was used as the probe, hybridizations were observed only with serovars 1 to 6, 8 to 11, and 21, whereas when DT1 was used, we observed hybridization with serovars 2, 3, 7, 12 to 20, and 23 to 25 (Fig. 4). Both *M. scrofulaceum* strains tested in the present study reacted with the DT6 probe, giving a weak hybridization signal with a 21-kb fragment, while they did not react with the DT1 probe (data not shown). In addition, with the enzyme used in the present study and with DT6 used as the probe, three different patterns were observed: two DNA fragments (4.2 and 1.8 kb long) were found to hybridize when the DNAs of serovars 1, 5, 6, 8, 9, 10, 11, and 21 were analyzed, two fragments (3 and 5 kb long) were obtained with the DNAs of serovars 2 and 3, while only one fragment of more than 5 kb was detected with the DNA of serovar 4. Only one fragment was found to hybridize with the DT1 probe. The size of this fragment was about 4.8 kb when the DNAs of serovars 2, 3, 7, 12 to 14, 16, 17, 20, and 23 to 25 were analyzed. When DNAs of serovars 15, 18, and 19 were analyzed, the length of the hybridized fragment ranged between 10 and 20 kb.

Neither DT1 nor DT6 was able to hybridize with the DNAs of serovars 22 and 26 to 28 under the conditions used in the present study.

Specificities of DT1- and DT6-derived primers in PCR experiments. In order to identify mycobacteria of the *M. avium-M. intracellulare* complex rapidly and without the need to prepare a large amount of DNA, as is required for Southern blot analysis, oligonucleotide pairs derived from the DT1 and DT6 sequences were used as primers in PCR experiments. Two pairs of primers, AV6-AV7 and IN38-IN41, were selected from the DT6 and DT1 sequences, respectively (Fig. 2), by using the computer program designed by Griffais et al. (8). The specificities of the selected primers were tested by using the organisms listed in Table 1; they included the type strains of 26 mycobacterial species as well as *T. paurometabolum*. The AV6 and AV7 primers were found to amplify a 187-bp fragment when the DNAs of *M. avium* and *M. avium* subsp. *paratuberculosis* were analyzed, while IN38 and IN41 amplified a 666-bp fragment when the DNAs of *M. avium* and *M. intracellulare* were used as templates. No DNA amplification was observed when we used these two pairs of primers and the other mycobacterial type strains.

In addition, total DNA purified from the 28 well-characterized serovars were amplified with these primers (Fig. 5). By using AV6 and AV7, amplification of the expected 187-bp fragment was observed for the strains carrying serotypes 1 to 6, 8 to 11, and 21; by using IN38 and IN41, amplification of the expected 666-bp fragment was observed for the strains carrying serotypes 2, 3, 7, 12 to 20, and 23 to 25. These results are in perfect correlation with those of the Southern blot analysis.

DISCUSSION

The current methods of identifying mycobacterial species that rely on biochemical tests are slow and may not be accurate, especially for *M. avium* and *M. intracellulare* species, and many laboratories report strains as members of the *M. avium* complex. This designation may include other related species. Mycolic acids represent valuable markers for the identification of slowly growing mycobacterial species (23). However, the mycolate patterns displayed by thin-layer chromatography are similar for both species and are shared by quite a few other species (4). No specific

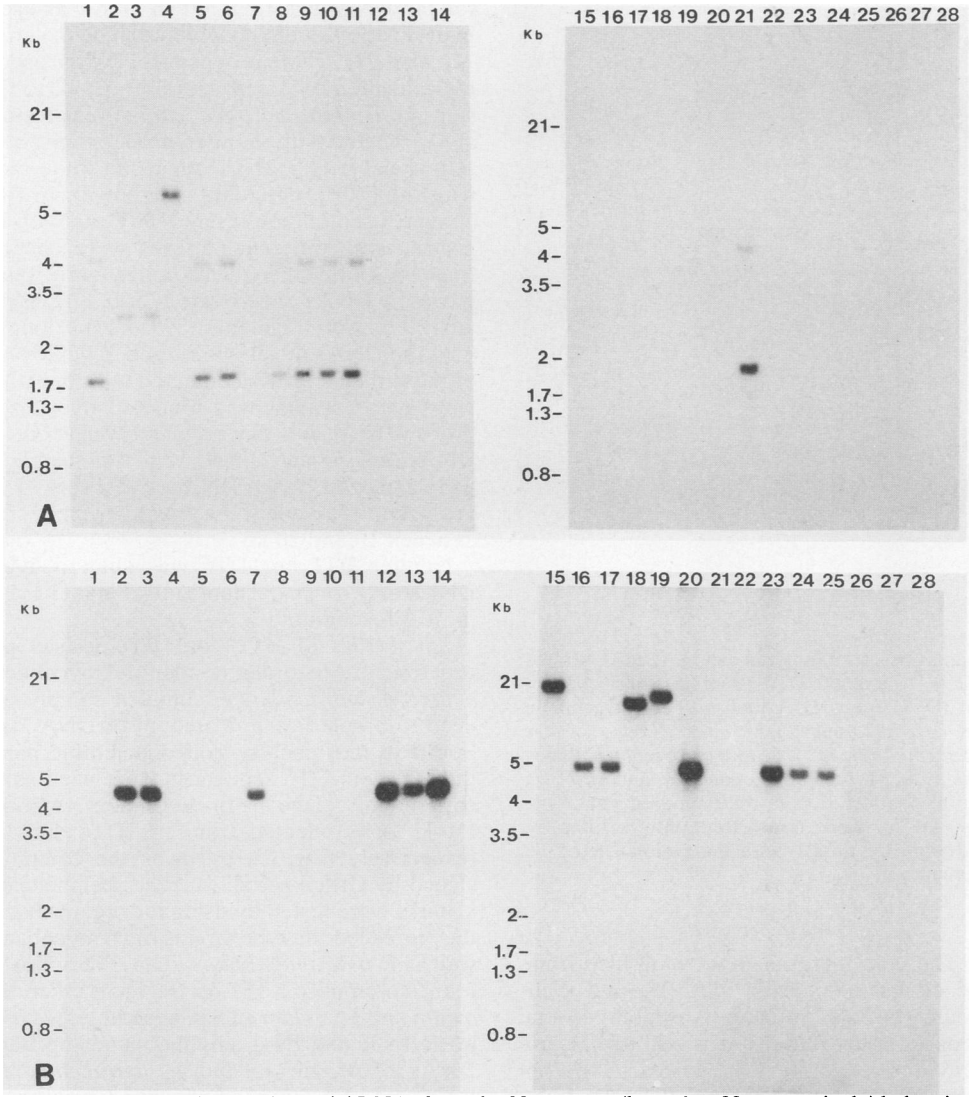

FIG. 4. Southern analysis of genomic mycobacterial DNAs from the 28 serovars (lanes 1 to 28, respectively) belonging to the *M. avium-M. intracellulare* complex. A total of 1 μg of genomic DNA was digested to completion with restriction enzyme *Pst*I. After electrophoresis, DNAs were transferred onto a filter by Southern blotting and were hybridized with the radiolabeled DT6 (A) and DT1 (B) fragments.

marker can be detected by gas chromatography (4). By these techniques, identification of *M. avium-M. intracellulare* is more the exclusion of other species rather than a true recognition and thus consists of an identification *a contrario*. Recently, it has been reported that high-pressure liquid chromatography (HPLC) provides profiles characteristic of each species, and these profiles allow the identification with 97.9 and 97.5% accuracies for *M. avium* and *M. intracellulare*, respectively (3).

Commercially available probes (Gen-Probe) have been successfully used for the identification of strains either on Löwenstein-Jensen or on liquid medium, e.g., BACTEC sediments. The latter procedure greatly contributes to a shortened time to identification and is easily applied in clinical laboratories (15).

Using these probes, Saito et al. (16) confirmed the results of Baess (1) on the basis of DNA-DNA hybridization studies and assigned serotypes 1 to 6, 8 to 11, and 21 to *M. avium* and serotypes 7, 12 to 20, and 25 to *M. intracellulare*. However, most strains of serotypes 23, 24, 26, and 28 did not hybridize with any of the probes but presented the MAC-specific α antigen (16). Even though Gen-Probe probes are directed against rRNA, some discrepancies are noted between rRNA sequencing and hybridization results. In particular, serotypes 7 and 18 have been reported to present rRNA sequences different from those of either *M. avium* or *M. intracellulare*, whereas these serotypes are both recognized by the Gen-Probe probe specific for *M. intracellulare* (2, 16). Moreover, Gen-Probe probes for *M. avium* or *M. intracellulare* do not allow for the detection of all clinical isolates of *M. avium* or *M. intracellulare*, as shown in different studies by using other DNA probes, serotyping, or mycolate analysis by HPLC (3, 7, 11, 12, 14).

Broadly, our results are in correlation with those described by Saito et al. (16), who proposed that *M. avium* species should include isolates of serovars 1 to 6, 8 to 11, and 21 and that *M. intracellulare* species should include isolates of serovars 7, 12 to 20, and 25. In addition, according to our

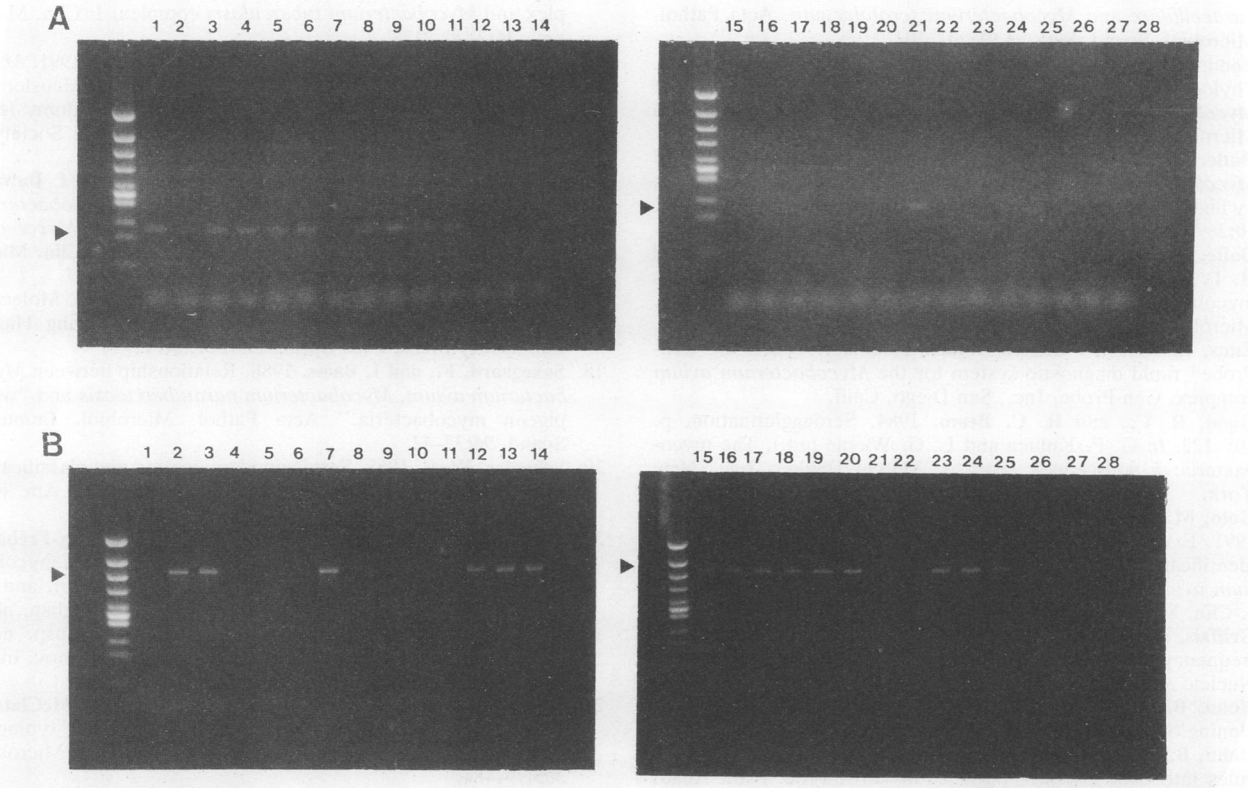

FIG. 5. Mycobacterial DNAs from serovars 1 to 28 (lanes 1 to 28, respectively) amplified by PCR by using the AV6-AV7 (A) and IN38-IN41 (B) oligonucleotides derived from fragments DT6 and DT1, respectively. Arrowheads indicate the 187-bp fragment (A) and the 666-bp fragment (B).

results, it seems that isolates of serovars 23 and 24 should be classified as *M. intracellulare* since these serovars hybridized with the DT1 probe but did not react with the DT6 probe.

Our results indicate that the DT1 and DT6 sequences described here are well suited for the identification of *M. avium* and *M. intracellulare* species. Indeed, DT1 and DT6 appear to be specific for members of the *M. avium-M. intracellulare* complex. Serovars 2, 3, 7, 12 to 20, and 23 to 25 showed clear positive hybridization with the DT1 probe and serovars 1 to 6, 8 to 11, and 21 hybridized with the DT6 probe. Thus, it appears that DT1 is specific for *M. intracellulare* serovars, while DT6 is specific for *M. avium* strains. However, the DT1 probe presents some overlap with the DT6 probe, since it also hybridizes with the DNAs of isolates of serotypes 2 and 3. The hybridization observed between DT1 and *M. avium* serovar 2 was expected, since the cosmid library was constructed with the total DNA isolated from this strain. More surprising was the hybridization obtained with the DNA of *M. avium* serovar 3, suggesting a closer genotypic homology between serovar 2 and serovar 3 than between serovar 2 and other *M. avium* serovars. In addition, the Southern blot analysis of the DNAs of serovars 2 and 3 provided the same hybridization pattern with the DT6 probe and this pattern was different from those obtained with the other *M. avium* serovars.

The DT6 probe gave a weak hybridization signal in Southern blot analysis with *M. scrofulaceum* DNA. However, with DT6-derived primers (AV6, AV7) no amplification was obtained with *M. scrofulaceum* DNA. The cross-hybridization observed with *M. paratuberculosis* and the DT6 probe was not surprising since this organism has been demonstrated to represent a subspecies of *M. avium*.

The results obtained by PCR with the DT1- and DT6-derived primers are in agreement with the results obtained by Southern blot analysis with the entire DT1 and DT6 probes. The DT1- and DT6-derived primers could thus be used in a PCR assay for the identification of *M. avium-M. intracellulare* strains at the species level. Differentiation of *M. avium* and *M. intracellulare* could be performed by using a double PCR test, since DT6-positive strains correspond to *M. avium*, DT1-positive strains correspond to *M. intracellulare*, and strains positive with both pairs of primers can be identified as *M. avium* serotype 2 or 3.

Technical improvements to the method, including the use of nonradioactive probes and a hybridization format, are being investigated to develop a test which could allow for the direct detection of these organisms in uncultured samples. Moreover, the epidemiological applications of the DT1 and DT6 probes are under investigation, as is evaluation of their use for the identification of clinical *M. avium* complex isolates.

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REFERENCES

1. **Baess, I.** 1983. Deoxyribonucleic acid relationships between different serovars of *Mycobacterium avium*, *Mycobacterium*

intracellulare and *Mycobacterium scrofulaceum*. Acta Pathol. Microbiol. Scand. Sect. B **91:**201–203.
2. **Böddinghaus, B., J. Wolters, W. Heikens, and E. Böttger.** 1990. Phylogenetic analysis and identification of different serovars of *Mycobacterium intracellulare* at the molecular level. FEMS Microbiol. Lett. **70:**197–204.
3. **Butler, W. R., L. Thibert, and J. Kilburn.** 1992. Identification of *Mycobacterium avium* complex strains and some similar species by high-performance liquid chromatography. J. Clin. Microbiol. **30:**2698–2704.
4. **Daffé, M., M. A. Lanéelle, C. Asselineau, V. Lévy-Frébault, and H. L. David.** 1983. Intérêt taxonomique des acides gras des mycobactéries: proposition d'une méthode d'analyse. Ann. Microbiol. (Inst. Pasteur) **134B:**241–256.
5. **Enns, R. K.** 1987. Clinical studies summary report: the Gen-Probe® rapid diagnostic system for the *Mycobacterium avium* complex. Gen-Probe, Inc., San Diego, Calif.
6. **Good, R. C., and R. C. Beam.** 1984. Seroagglutination, p. 105–122. *In* G. P. Kubica and L. G. Wayne (ed.), The mycobacteria: a sourcebook, Part A. Marcel Dekker, Inc., New York.
7. **Goto, M., S. Oka, K. Okuzumi, S. Kimura, and K. Shimada.** 1991. Evaluation of acridinium-ester-labeled DNA probes for identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-Mycobacterium intracellulare* complex in culture. J. Clin. Microbiol. **29:**2473–2476.
8. **Griffais, R., P. M. André, and M. Thibon.** 1991. K-tuple frequency in the human genome and polymerase chain reaction. Nucleic Acids Res. **19:**3887–3891.
9. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11:**291–298.
10. **Hohn, B., and K. Murray.** 1977. Packaging recombinant molecules into bacteriophage particles *in vitro*. Proc. Natl. Acad. Sci. USA **74:**3259–3263.
11. **Lebrun, L., F. Espinasse, J. D. Poveda, and V. Vincent Lévy-Frébault.** 1992. Evaluation of nonradioactive DNA probes for identification of mycobacteria. J. Clin. Microbiol. **30:**2476–2478.
12. **Lim, S. D., J. Todd, J. Lopez, E. Ford, and J. M. Janda.** 1991. Genotypic identification of pathogenic *Mycobacterium* species by using a nonradioactive oligonucleotide probe. J. Clin. Microbiol. **29:**1276–1278.
13. **McFadden, J. J., P. D. Butcher, R. Chiodini, and J. Hermon-Taylor.** 1987. Determination of genome size and DNA homology between unclassified mycobacterium species isolated from patients with Crohn's disease and other mycobacteria. J. Gen. Microbiol. **133:**211–213.
14. **Musial, C. E., L. S. Tice, L. Stockman, and G. D. Roberts.** 1988. Identification of mycobacteria from culture by using the Gen-Probe rapid diagnostic system for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. J. Clin. Microbiol. **26:**2120–2123.
15. **Roberts, G. D., E. W. Koneman, and Y. K. Kim.** 1991. *Mycobacterium*, p. 304–339. *In* A. W. Balows, W. J. Hausler Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society of Microbiology, Washington, D.C.
16. **Saito, H., H. Tomioka, K. Sato, H. Tasaka, and D. J. Dawson.** 1990. Identification of various serovar strains of *Mycobacterium avium* complex by using DNA probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. J. Clin. Microbiol. **28:**1694–1697.
17. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
18. **Saxegaard, F., and I. Baess.** 1988. Relationship between *Mycobacterium avium*, *Mycobacterium paratuberculosis* and "wood pigeon mycobacteria." Acta Pathol. Microbiol. Immunol. Scand. **96:**37–42.
19. **Schaefer, W. B.** 1965. Serologic identification and classification of the atypical mycobacteria by their agglutination. Am. Rev. Respir. Dis. **92**(Suppl.)**:**85–93.
20. **Thorel, M. F., M. Krichevsky, and V. Vincent Lévy-Frébault.** 1990. Numerical taxonomy of mycobactin-dependent mycobacteria, emended description of *Mycobacterium avium*, and description of *Mycobacterium avium* subsp. *avium* subsp. nov., *Mycobacterium avium* subsp. *paratuberculosis* subsp. nov., and *Mycobacterium avium* subsp. *silvaticum* subsp. nov. Int. J. Syst. Bacteriol. **40:**254–260.
21. **Tsang, A. Y., J. C. Denner, P. J. Brennan, and J. K. McClatchy.** 1992. Clinical and epidemiological importance of typing of *Mycobacterium avium* complex isolates. J. Clin. Microbiol. **30:**479–484.
22. **Tsang, A. Y., I. Drupa, M. Goldberg, J. K. McClatchy, and P. J. Brennan.** 1983. Use of serology and thin-layer chromatography for the assembly of an authenticated collection of serovars within the *Mycobacterium avium-Mycobacterium intracellulare-Mycobacterium scrofulaceum* complex. Int. J. Syst. Bacteriol. **33:**285–292.
23. **Vincent Lévy-Frébault, V., and F. Portaels.** 1992. Proposed minimal standards for the genus *Mycobacterium* and for description of new slowly growing *Mycobacterium* species. Int. J. Syst. Bacteriol. **42:**315–323.
24. **Wayne, L. G., and H. A. Sramek.** 1992. Agents of newly recognized or infrequently encountered mycobacterial diseases. Clin. Microbiol. Rev. **5:**1–25.
25. **Yoshimura, H. H., and D. Y. Graham.** 1988. Nucleic acid hybridization studies of mycobactin-dependent mycobacteria. J. Clin. Microbiol. **26:**1309–1312.