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A Novel Insertion Element from *Mycobacterium avium*, IS*1245*, Is a Specific Target for Analysis of Strain Relatedness

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The insertion sequence IS*1245* is a novel mycobacterial repetitive element identified in *Mycobacterium avium*. It encodes a transposase which exhibits a 64% amino acid similarity with IS*1081*, an insertion element present in the *M. tuberculosis* complex. The host range of IS*1245* appears limited to *M. avium* as this element was not identified in *M. intracellulare* or in any other of 18 mycobacteria species tested. When IS*1245* was used for restriction fragment length polymorphism (RFLP) analysis, human isolates characteristically presented a high number of copies (median, 16; range, 3 to 27) and a diversity of RFLP patterns comparable to that found by pulsed-field gel electrophoresis. Isolates from nonhuman sources differed both in number of copies and in RFLP pattern diversity: while swine isolates shared the characteristics of human strains, those from several avian sources exhibited a very low copy number of IS*1245* and appeared clonal on the basis of RFLP.

A better understanding of the biology of *Mycobacterium avium* will require a comprehensive evaluation of animal and environmental reservoirs from which humans acquire the infection, routes of transmission, and means to identify virulent clones. In addition, a definite separation of *M. avium* from other members of the *M. avium-M. intracellulare* complex and a more precise delineation of subgroups within the *M. avium* species will be desirable to put an end to a lasting taxonomic "gray zone" which obscures the interpretation of medical and veterinary studies of *M. avium* infection.

A number of laboratory techniques have been applied, with different degrees of success, to these purposes (15). In particular, significant experience with serotyping (23) and multilocus enzyme electrophoresis (28) has been accumulated, more recently including several molecular tools: restriction fragment length polymorphism (RFLP) and hybridization to specific probes (12, 21, 22), sequencing or restriction analysis of diverse genetic regions (8, 10, 16), and pulsed-field gel electrophoresis (PFGE) (1, 20). Each technique allows for different levels of species, subspecies, and strain characterization within the *M. avium-M. intracellulare* complex. PFGE is now considered the gold standard for determining strain relatedness (19), and it has helped to establish water as a definite reservoir for human infection (27) and to determine the degree of diversity among strains in a community (4).

Insertion sequences present several characteristics that make them of great interest for epidemiological evaluation, as well as for phylogenetic and taxonomic studies. Depending on the degree of mobility and the copy number of the insertion element, DNA fingerprints are generated upon Southern blot and hybridization and can be used to infer strain relatedness and, possibly, epidemiological linkage. Many elements belong to families of insertion sequences and transposons, and their comparative analysis can shed light on evolutionary relationships (2). They are frequently species specific; thus, insertion sequences can be used for species identification.

To date, 11 insertion elements have been described in mycobacteria (13, 18). Of these, four elements have been identified in the *M. avium-M. intracellulare* complex: IS*900* in *M. avium* subsp. *paratuberculosis*, IS*901* and IS*1110* in *M. avium*, and IS*1141* in *M. intracellulare.*

In this report, we describe a novel insertion element, IS*1245*, which is present in high copy number in the chromosomes of *M. avium* strains isolated from human sources.

MATERIALS AND METHODS

Primers and probe. IS*1245* was initially identified as an *M. avium* genomic fragment which generated complex RFLP patterns when used as a probe. Its complete nucleotide sequence has been submitted to GenBank under accession number L33879. On the basis of the sequence of IS*1245*, two primers were synthesized: P1 (5′-GCCGCCGAAACGATCTAC) and P2 (5′-AGGTGGC GTCGAGGAAGAC), at positions 135 to 152 and 543 to 561, respectively.

PCR. The two primers were used in PCR to define the host range of IS*1245* among 19 different mycobacteria (Table 1) and to generate a 427-bp probe used in hybridization experiments. In addition, 82 *M. avium* strains from different sources were investigated. These included 8 *M. avium* laboratory reference strains, 58 human isolates (48 from Switzerland and 10 from other countries, i.e., Belgium, Holland, Honduras, and Australia), and 16 animal isolates (five from poultry, five from pigs, one each from a parrot, dog, buffalo, and lizard, one *M. avium* subsp. *paratuberculosis*, and one *M. avium* subsp. *silvaticum*).

PCR was performed as previously described (22), under the following conditions: 30 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C and one final extension cycle of 10 min at 72°C.

Southern blot and hybridization. Mycobacterial strains were grown for 10 to 14 days in 5 ml of Middlebrook 7H9 broth supplemented with 10% oleic acid–albumin–glucose–catalase (Difco Laboratories) and 0.05% Tween 80. Cultures were centrifuged, resuspended in 0.85% NaCl, and inactivated by heat (30 min at 80°C). Thereafter, bacterial DNA was extracted (25), cut with *Pvu*II, and electrophoresed on a 0.7% agarose gel. DNA fragments were transferred by Southern blotting onto a nylon membrane and hybridized to the PCR-generated digoxigenin-labelled probe as previously described (9). Targets were detected by chemiluminescence (DIG luminiscent detection kit; Boehringer Mannheim, Mannheim, Germany).

PFGE. *M. avium* strains (n = 38) included in the study were subjected to PFGE, using *Ase*I as described previously (4). The discriminatory abilities of PFGE and RFLP-IS*1245* were compared by applying Simpson's index of diversity (14).

Sequence analysis. A GCG software package (Genetics Computer Group, Madison, Wis.) was used for comparison and alignment of IS*1245* with members of the IS*256* family. Phylogenetic trees were constructed by using PROTDIST and FITCH from PHYLIP 3.5 (J. Felsenstein, University of Washington).

RESULTS

Characteristics of IS*1245*. IS*1245* is a 1,313-bp element delimited by two imperfect inverted repeats with an open reading frame encoding a putative transposase (Fig. 1A). The IS*1245* transposase exhibits a high degree of homology (64% amino

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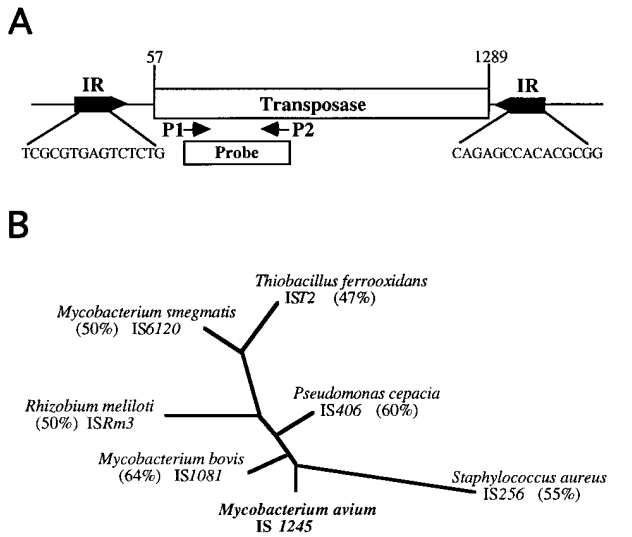

FIG. 1. Structure of IS*1245* and relationship to other insertion elements. (A) Schematic representation of the 1,313-bp element. Shown are the inverted repeats (IR), the primers used to generate probes for RFLP and PCR, and the beginning and end of the open reading frame encoding a putative transposase. (B) Phylogenetic tree of the seven members of the IS*256* family of insertion elements. Indicated are the percent amino acid similarities between IS*1245* and the other elements. The unrooted tree was created from distance matrix files by Fitch-Margoliash analysis.

acid similarity) with *M. bovis* IS*1085*. Both elements belong to the *Staphylococcus aureus* IS*256* family of insertion sequences (Fig. 1B).

Host range of IS*1245*. The presence of IS*1245* in species of the *Mycobacterium* genus (Table 1) was investigated by amplification of a 427-bp target sequence within IS*1245*. Results of PCR were confirmed by hybridization of total genomic DNA with the IS*1245* probe. Only mycobacteria included in the *M. avium* group (*M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, and *M. avium* subsp. *silvaticum*) contained the element.

RFLP analysis of *M. avium* strains. Human isolates of *M. avium* exhibited an elevated number of IS*1245* copies (median, 16; range, 3 to 27) which resulted in highly diverse RFLP patterns. Results were in general agreement with those obtained by PFGE analysis (Fig. 2). When 38 isolates were investigated by both techniques, strains could be assigned to 27 and 22 different groups by RFLP-IS*1245* and PFGE, with discriminatory indices (14) of 0.97 and 0.95, respectively. The most significant discrepancy corresponded to a set of six isolates with identical PFGE patterns and highly related but not identical RFLP-IS*1245* fingerprints. Pattern differences in the positions of 3 to 5 of the approximately 27 IS*1245* copies were seen (Fig. 2, lanes 6 and 7). Two separate isolates from the same patient before and after the development of resistance to clarithromycin (Fig. 2, lanes 4 and 5) and 11 isolates representing an event of laboratory contamination exhibited the same RFLP patterns.

One human isolate contained a single copy of IS*1245*. Sequencing of its 16S rRNA region demonstrated a sequence similar to that reported for *M. avium* subsp. *paratuberculosis* (16). However, this strain did not possess the element IS*900*, described as characteristic for this subspecies (26).

DNA from *M. avium* strains isolated from animals revealed significant differences upon hybridization with the IS*1245* probe. Swine isolates, like human strains, exhibited a high copy

TABLE 1. Laboratory strains and isolates investigated

Organism	No. of strains	Laboratory reference strain(s)
M. avium	80	ATCC 15769, 25291, 35713, 35714, 35715, 35716, 35718[a]; 140031.0005[b]
M. avium subsp. *paratuberculosis*	1	Institute of Veterinary Microbiology, University of Berne
M. avium subsp. *silvaticum*	1	Institute of Medical Microbiology, University of Berne
M. intracellulare	9	ATCC 35770,[a] 140031.0001[b]
M. tuberculosis	4	H37Rv 14001 0001[b]
M. bovis	3	14002. 0001,[c] BCG[d]
M. smegmatis	2	mc^2 6[c]
M. kansasii	2	NCTC 10268[e]
M. fortuitum	3	ATCC 6841[a]
M. terrae	3	DSM 10111.68[f]
M. malmoense	2	NCTC 11298[e]
M. chitae	1	NCTC 10485[e]
M. chelonae	2	
M. fallax	1	
M. genavense	1	
M. xenopi	1	
M. simiae	1	
M. marinum	1	
M. gordonae	1	
M. scrofulaceum	1	
M. nonchromogenicum	1	

[a] American Type Culture Collection, Rockville, Md.
[b] Institut Pasteur, Paris, France.
[c] W. R. Jacobs, Jr.
[d] Statens Seruminstitut, Copenhagen, Denmark.
[e] National Collection of Type Cultures, London, England.
[f] Deutsche Stammsammlung für Mikroorganismen, Braunschweig, Germany.

number of IS*1245* (more than eight elements) and highly polymorphic RFLPs (Fig. 3, lane 3). In contrast, a characteristic two-band pattern was identified in all American Type Culture Collection reference strains of chicken origin investigated, in strains from three chickens and a duck from different Swiss farms, and in strains from a goose and a lizard both from a Belgian zoo. This unique pattern was also identified in three additional reference strains of bovine (ATCC 35715), human

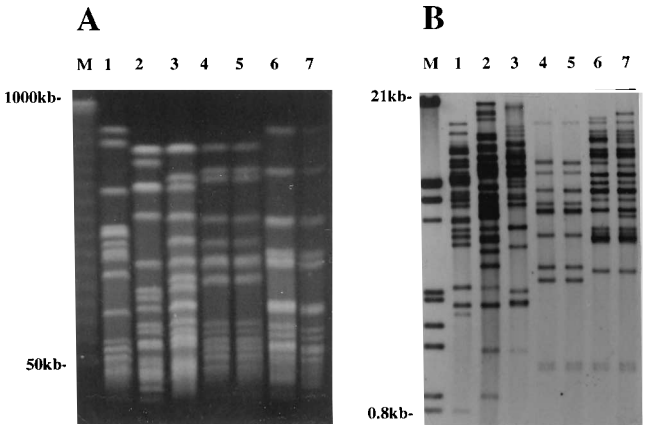

FIG. 2. Comparison of PFGE (A) and RFLP-IS*1245* (B) for discrimination of strain relatedness among human isolates. Shown are isolates with unique patterns (lanes 1 to 3), isolates before and after developing resistance to clarithromycin (lanes 4 and 5), and isolates from two patients which, despite identical PFGE patterns, present a shift of several IS*1245* copies in an otherwise related RFLP fingerprint. M, molecular size marker.

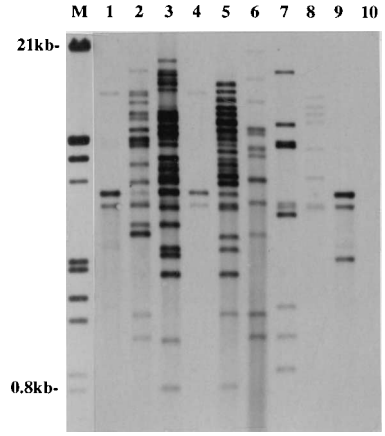

FIG. 3. RFLP-IS*1245* patterns of *M. avium* from human and animal sources: *M. avium* reference strain ATCC 25291 (lane 1) and isolates from a human (lane 2), a pig (lane 3), a chicken (lane 4), a parrot (lane 5), a bovine (lane 6), and a dog (lane 7); *M. avium* subsp. *paratuberculosis* (lane 8); and *M. avium* subsp. *silvaticum* (lane 9). Lane 10, reference strain of *M. intracellulare*. M, molecular size marker.

(ATCC 35718), and porcine (Pasteur 140031.0005) origin and in two clinical isolates from Belgium. A related three-band pattern was also found in an isolate from a wood pigeon which was identified as *M. avium* subsp. *silvaticum* (Fig. 3, lane 9). Other animal isolates studied included strains from a dog (4 bands), a parrot (23 bands), and a buffalo (16 bands), bovine reference strain ATCC 35716 (9 bands), and a laboratory strain of *M. avium* subsp. *paratuberculosis* (7 bands) (Fig. 2).

DISCUSSION

The new repetitive element IS*1245* adds to the growing list of mycobacterial insertion sequences (13, 18). It belongs to a class of related insertion elements which includes *S. aureus* IS*256* (5), *Thiobacillus ferrooxidans* IS*T2* (31), *Rhizobium meliloti* IS*Rm3* (29), *Pseudomonas cepacia* IS*406* (30), and the two mycobacterial elements IS*6120* from *M. smegmatis* (11) and IS*1081* from *M. bovis* (7).

A significant homology between IS*1245* and *M. bovis* IS*1081* was found, both in the structure of the inverted repeats and in the deduced amino acid sequence of the encoded putative transposase. *M. bovis* IS*1081* generates a minimal polymorphism when used as a probe for RFLP (6), which suggests a limited mobility of the element in the *M. tuberculosis* complex or a high degree of insertion specificity. In contrast, IS*1245* is present in multiple copies and generates considerable polymorphism. Thus, it can be inferred that the IS*1245* element has a marked insertion promiscuity, high mobility, or both, which would make it an attractive tool for transposon mutagenesis studies (18).

The presence of multiple copies of IS*1245* in all human *M. avium* clinical isolates tested prompted us to investigate the use of this element for epidemiological purposes, much as has been done with IS*6110* from *M. tuberculosis* (24). RFLP using IS*1245* appeared to have a discriminatory power for strain differentiation comparable to that achieved with PFGE (14). However, several isolates sharing an identical PFGE pattern were found to differ in the positions of up to five of the approximately 27 copies of IS*1245*. Whether these findings represent better performance of RFLP-IS*1245* over PFGE for the analysis of strain relatedness or reflect a limited stability of the insertion element, which would make it a suboptimal target for the purpose of epidemiological studies, has not yet been established. IS*1245* has the relative advantage over PFGE and some of the previously used probes (12) of being species specific; most importantly, it does not hybridize with *M. intracellulare*. The other known *M. avium* insertion elements (IS*900* and IS*901*) have not been useful for epidemiological studies because of limited polymorphism (17, 26). IS*1110*, a new insertion element related to the IS*900* family, is reported to have a significant degree of mobility, with RFLP patterns more diverse than those seen with IS*900* and IS*901* (13).

When we extended our experience to the testing of nonhuman *M. avium* isolates, a striking difference in the number of IS*1245* elements was seen: strains from poultry, including several American Type Culture Collection reference strains of chicken origin, displayed a unique two-band pattern on RFLP. Only 2 of 58 human isolates, both from a respiratory source and collected in Belgium, and three other reference strains (from putative bovine, human, and pig sources) exhibited such a pattern. A related three-band pattern was also identified in an isolate of *M. avium* subsp. *silvaticum*. These findings could indicate the existence of a widely spread *M. avium* subgroup of related strains which, importantly, are pathogenic for poultry (3). In contrast, the number of copies and the diversity of RFLPs among isolates from swine and other animal sources appeared similar to those of human isolates.

In summary, RFLP using IS*1245* represents a tool for the analysis of *M. avium* strain relatedness. As it appears to be species specific, it will aid in structuring the complex taxonomy of the *M. avium-M. intracellulare* complex. Finally, because it is a high-copy-number, potentially mobile element absent in the *M. tuberculosis* complex, IS*1245* may be of interest in transposon mutagenesis.

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ADDENDUM IN PROOF

Since completing this work, we have investigated the insertion sites of IS*1245* and shown the inverted repeats to be of greater length than previously estimated. The corresponding sequence submission to GenBank (accession number L38879) has been updated.

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