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IS*6110* Homologs Are Present in Multiple Copies in Mycobacteria Other than Tuberculosis-Causing Mycobacteria

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We have previously demonstrated homology between a 181-bp fragment of IS*6110* and DNA from mycobacteria other than tuberculosis-causing mycobacteria (MOTT). Genomic DNA from 14 strains of MOTT was digested with *Pvu*II and was hybridized with a probe derived from the 181-bp fragment and the INS1/INS2 international standard probe at high stringency. Multiple banding patterns were obtained from isolates of *M. avium-M. intracellulare*, *M. fortuitum*, *M. kansasii*, and *M. malmoense*. Differences in the banding patterns between and within species were obtained. This suggests that mycobacteria possess a family of IS*3*-like elements. The species of isolates suspected of being *M. tuberculosis* must be carefully determined before IS*6110* restriction fragment length polymorphism analysis, and caution must be used in designing and evaluating diagnostic PCR tests based on this element.

Insertion sequences (ISs) are mobile genetic elements 0.8 to 2.5 kb in length with an inverted repeat at each end and an open reading frame which codes for a transposase, mediating their mobility (12, 13). They differ from transposons because they only carry genes necessary for their own replication. ISs may have a role to play in the introduction of biological diversity within a genus. Understanding of the biology of ISs is fundamental to our understanding of the biology and evolution of the *Mycobacterium* genus.

More than 16 different ISs belonging to five IS families have been reported in mycobacteria (13). Mycobacterial ISs are used to study the epidemiology of *M. tuberculosis* and *M. avium-M. intracellulare* (17), and it is expected that improved understanding of tuberculosis transmission will come as a result of the application of IS typing methods. Many such studies are now under way throughout the world (6, 7).

The IS IS*6110* is a member of the IS*3* family. Members of this family are the most widely spread group of bacterial ISs, being found in more than 24 different gram-positive and gram-negative genera (1). Most strains of *M. tuberculosis* have between 8 and 15 copies (9, 13), although some, found throughout the world, have only 1 copy and others lack IS*6110* completely (18).

The IS IS*6110* has previously been said to be specific to *M. tuberculosis* (17). Using a PCR method, we have shown that mycobacteria of other species including *M. avium-M. intracellulare*, *M. fortuitum*, *M. kansasii*, *M. xenopi*, and *M. malmoense* possess a sequence homologous to a central 181-bp fragment of IS*6110*, a result which was confirmed by hybridization of genomic DNA from these species (10) (Fig. 1). We now report further studies of cross-hybridization of IS*6110* sequences and mycobacteria other than tuberculosis-causing mycobacteria (MOTT).

MATERIALS AND METHODS

Bacterial isolates. Isolates were obtained from the clinical microbiology diagnostic service of the Royal Free Hospital, London, United Kingdom. Determination of the species of the isolates was confirmed by the Mycobacterial Reference Unit, Dulwich, United Kingdom. Three isolates each of *M. avium-M. intracellulare* and *M. malmoense* and four isolates each of *M. fortuitum* and *M. kansasii* were studied.

Southern hybridization. DNA was extracted by standard methods (10), and the samples were digested with *Pvu*II restriction endonuclease and separated by agarose gel electrophoresis by the international standard typing method for *M. tuberculosis* (17). The probes used were PCR amplimers derived from reaction mixtures with H37Rv DNA as a template and the following primer sets: primers Tb670 (5′-AGT TTG GTC ATC AGC C-3′) and Tb505 (5′-ACG ACC ACA TCA ACC-3′) (181-bp fragment) (10) and primers INS1 (5′ CGT GAG GGC ATC GAG GTG GC 3′) and INS2 (5′-GCG TAG GCG TCG GTG ACA AA-3′) (INS1/2 restriction fragment length polymorphism [RFLP] probe) (14) (Fig. 1). The probes were labelled and detected by chemiluminescence procedures, as recommended by the manufacturers (Amersham International, Amersham, United Kingdom). Hybridization was performed at 50°C, and the final wash was with 0.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 50°C.

RESULTS

Multiple banding patterns were obtained with the probe derived from the 181-bp probe described above and the INS1/2 probe for DNA derived from *M. avium-M. intracellulare*, *M. fortuitum*, *M. malmoense*, and *M. kansasii*. These results are illustrated in Fig. 2 to 5. Although the patterns were broadly similar, for each isolate there were differences between the banding patterns obtained with the 181-bp probe and those obtained with the INS1/2 probe.

Three isolates of *M. avium-M. intracellulare* were studied, and minor differences between all three were demonstrated. All three isolates of *M. malmoense* demonstrated divergent banding patterns. For *M. fortuitum*, the patterns for isolates three and four were similar and markedly different from those for isolates one and two. The patterns for two of the *M. kansasii* isolates (isolates two and four) were similar, but those for the other two *M. kansasii* isolates were clearly different from those for isolates two and four.

DISCUSSION

Our previous study demonstrated the presence of a homologous sequence by PCR, and this was confirmed by dot blot hybridization of genomic DNA (10). Despite the hybridization data, other investigators have suggested that our results may have been due to PCR contamination (8). In this study PCR

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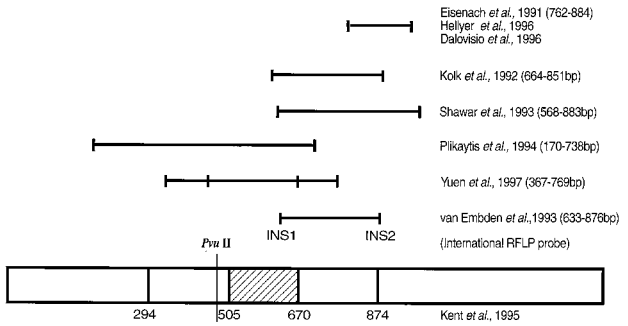

FIG. 1. Schematic drawing of IS*6110* showing locations of sequences amplified by published PCR protocols and the international standard RFLP probe. The references listed on the right are references 4, 8, 2, 11, 16, 15, 19, 17, and 10 (from top to bottom, respectively) in the References section of this report.

was only used to prepare probes, homology was demonstrated by Southern hybridization of genomic DNA, and a higher stringency than that of the international standard method was used, implying that the sequences have a high degree of homology for IS*6110*. Homologous sequences are present in multiple copies in all of the species of *Mycobacterium* tested, as can be seen in Fig. 2 to 5.

If the multiple bands are due to the presence of an IS, then the distribution that we have shown in the genus could have arisen by movement of IS*6110* between species. Hellyer et al. (8) reported the use of a PCR amplifying the region of IS*6110* from positions 762 to 865 on DNA derived from MOTT provided by our laboratory. No positive results were obtained, although the amount of DNA used in the experiments was not quantified. These data suggest either that there is a lower degree of homology or that the homology that we have demonstrated does not extend to the region covered by their primers (4, 8). We believe that it is more likely that mycobacteria may possess a family of IS*3* sequences which are closely related. A new IS, IS*1137*, in *M. smegmatis* has been identified and sequenced recently. It shows the characteristics of members of the IS*3* family and areas of homology with IS*6110* (5), lending support to the idea that there is a family of IS*3* sequences within the genus *Mycobacterium.*

A practical consequence of our data is that prior to RFLP analysis by the international standard protocol, the species of the isolates must be determined. MOTT produce RFLP patterns similar to those of *M. tuberculosis* that may be erroneously incorporated into a database. Our initial observation of

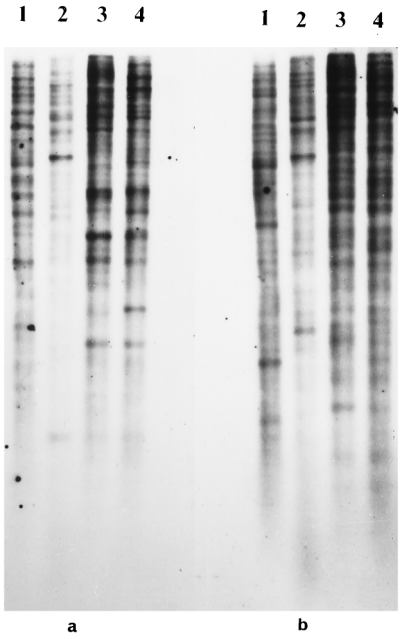

FIG. 3. Four clinical isolates (lanes 1 to 4) of *M. fortuitum* hybridized with 181-bp IS*6110* probe (a) and the international INS1/2 probe (b).

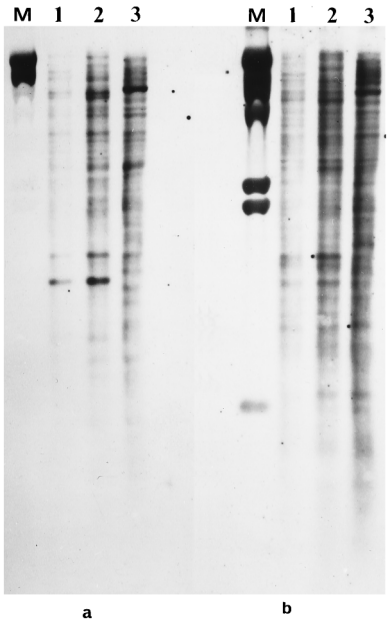

FIG. 2. Three clinical isolates (lanes 1 to 3) of *M. avium-M. intracellulare* hybridized with 181-bp IS*6110* probe (a) and the international INS1/2 probe (b). Lanes M, bacteriophage lambda molecular size marker.

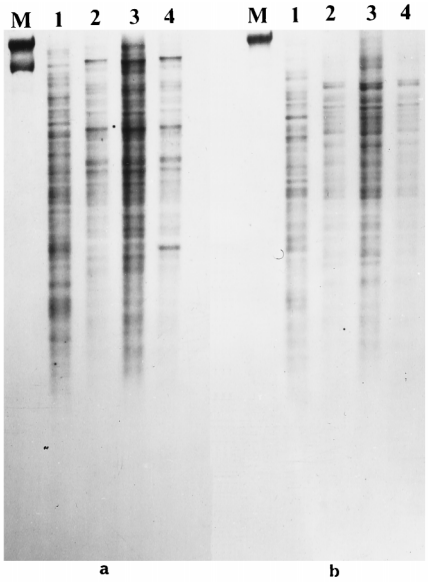

FIG. 4. Four clinical isolates (lanes 1 to 4) of *M. kansasii* hybridized with 181-bp IS*6110* probe (a) and the international INS1/2 probe (b). Lanes M, bacteriophage lambda molecular size marker.

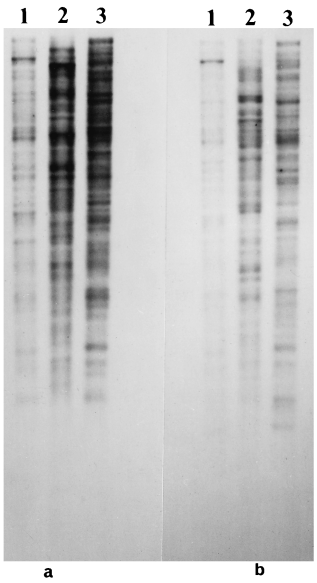

FIG. 5. Three clinical isolates (lanes 1 to 3) of *M. malmoense* hybridized with 181-bp IS*6110* probe (a) and the international INS1/2 probe (b).

homology in non-*M. tuberculosis* species was made when an organism which had a three-band pattern by the international standard RFLP method was subsequently shown to be *M. fortuitum*. In a review of our *M. tuberculosis* IS*6110* fingerprint database, in which isolates are ascribed RFLP patterns while the species designation is pending, 10.5% (9 of 85) were later confirmed to be MOTT (data not shown).

Because all of the non-*M. tuberculosis* isolates studied here produced a multiple banding pattern, it may be that this approach could be used to type non-*M. tuberculosis* isolates as a preliminary method before more specific techniques are used. We have examined a small number of isolates from our laboratory and found significant differences in banding patterns. The clinical utility of this approach can only be evaluated by the prospective studies which are now under way in our hospital.

IS*6110* remains a popular target sequence for diagnostic PCRs for *M. tuberculosis*. Our data extend the area within which care must be taken in the design and evaluation of primer pairs. Although some primer pairs have good records of specificity, it is perhaps significant that increasing amounts data regarding the low specificity of IS*6110*-based PCR methods are emerging (3, 14). The IS*6110*-based PCR of Eisenach et al. (4) was used in a recent clinical evaluation (2). They found in a study of 428 specimens 116 which subsequently grew MOTT. Three of these specimens were positive by IS*6110* PCR. A recent evaluation of IS*6110*-based methods in nine laboratories from France demonstrated false-positive reactions with an average rate of 7%. In that study a clear relationship to non-*M. tuberculosis* isolates or other organisms could not be drawn, although a majority of false-positive reactions were obtained for specimens which contained MOTT (3). The investigators conclude that the results of their study suggest "that PCR using IS*6110* as a target for DNA amplification is neither very sensitive nor really specific for the detection of tuberculosis" (3).

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