***Mycobacterium xenopi* IS1395, a novel insertion sequence expanding the IS256 family**

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An insertion sequence (IS) of *Mycobacterium xenopi* has been isolated and sequenced. This 1323 bp element, designated IS1395, is present in up to 18 copies in the *M. xenopi* genome and may be harboured in an *M. xenopi* extra-chromosomal element. It encodes a putative transposase of 415 amino acids which displays sequence homology to the *Staphylococcus aureus* IS256 family. Members of this class of elements have been described in the genus *Mycobacterium* – for example IS1081 is present in the *M. tuberculosis* complex, IS1245-IS1311 in *M. avium*, and IS6120 in *M. smegmatis*; these elements exhibit an 89%, 45% and 16% amino acid identity with IS1395, respectively. Investigation of the host range of IS1395 by Southern blot analysis revealed additional IS1395-related repeated sequences in *M. goodii* and *M. celatum*. Moreover, IS1395 represents a useful epidemiological tool for *M. xenopi* strain typing as it provides a diversity of restriction fragment length polymorphism patterns.

Keywords: *M. xenopi*, IS1395, IS256 family, RFLP typing

INTRODUCTION

Mycobacterium xenopi was first isolated from a toad (Schwabacher, 1959), but was not recognized as a human pathogen until 1965 (Marks & Schwabacher, 1965). Along with *M. avium* and *M. kansasii*, *M. xenopi* represents one of the commonest agents of mycobacterial pulmonary infections other than tuberculosis (Wolinsky, 1992). All of these organisms may colonize individuals without causing any damage; disease is usually encountered in patients presenting pre-existing lung disorders. Clinical, radiological and histopathological presentation is similar to that of tuberculosis and bacteriological findings are the only way of determining the causal agent. Unlike tuberculosis, notification of cases due to these potentially pathogenic mycobacteria is not mandatory and the actual incidence of related diseases is difficult to estimate. Moreover, the relative incidence of infections involving mycobacterial species varies significantly according to geographical area. The highest percentage of pulmonary infections due to *M. xenopi* is encountered in Western

Europe and the lowest in Japan and the USA. Rare extrapulmonary cases have been described in non-immunocompromised patients, whereas disseminated infections have been diagnosed in patients either transiently or chronically immunocompromised (Wayne & Sramek, 1992). Like other potentially pathogenic mycobacteria, *M. xenopi* is widely present in the environment, especially in tap water. Whereas clusters of cases tend to occur, human-to-human transmission has never been demonstrated and environmental sources of infection are likely. However, detailed investigation of animal and environmental reservoirs, as well as the determination of transmission routes and infection modes, requires precise strain-typing techniques.

There are two widely used molecular epidemiological methods for characterization of strains by genomic fingerprinting: pulsed-field gel electrophoresis (PFGE), and restriction fragment length polymorphism (RFLP) upon hybridization to specific probes. Ribotyping, a method of general use for bacteria, cannot be used in mycobacteria as they contain only one or two genes encoding rRNA (Bercovier *et al.*, 1985). The insertion sequence (IS) IS6110, specific for tubercle bacilli, has been demonstrated to be a valuable epidemiological tool to establish strain relatedness relying on RFLP patterns (van Embden *et al.*, 1992). Several other repeated DNA sequences have been described in mycobacteria, some

The GenBank accession number for the IS1395 sequence reported in this paper is U35051.

Abbreviations: IS, insertion sequence; IR, inverted repeat; DR, direct repeat.

providing genetic markers for species identification or strain differentiation (MacAdam *et al.*, 1994; Poulet & Cole, 1995; van Soolingen *et al.*, 1993). Moreover, analysis of IS structures may allow determination of evolutionary relationships between species and contribute to phylogenetic and taxonomic studies.

IS1081, isolated from *M. bovis*, is present in five to seven copies in strains of the *M. tuberculosis* complex and revealed very few RFLPs (van Soolingen *et al.*, 1992, 1993; Collins & Stephens, 1991; Collins *et al.*, 1993). An investigation of its host range showed cross-hybridization between *M. bovis* IS1081 and an unidentified repeated element in *M. xenopi* (Collins *et al.*, 1993; Collins, 1994).

In the present study, we screened a partial *M. xenopi* genomic library with an IS1081 probe. A clone was selected from which a new IS was isolated, designated IS1395, the molecular properties of which are presented here. Furthermore, the usefulness of this repetitive element for molecular strain typing within the species *M. xenopi* was analysed.

METHODS

Mycobacterial isolates. *M. xenopi* strains used in this study were isolated from clinical samples from different patients (Table 1).

Strains of eleven other mycobacterial species, *M. avium*, *M. bovis*, *M. intracellulare*, *M. marinum*, *M. gordonae*, *M. smegmatis*, *M.*

Table 1. *M. xenopi* strains used in the RFLP and PFGE studies

Strain no.	Patient	Source	Reference
950291	A	Bronchial wash	Our laboratory
950312	B	Sputum	Our laboratory
950703	C	Sputum	Our laboratory
950652	D	Gastric aspirate	Our laboratory
950358	E	Gastric aspirate	Our laboratory
950617	F	Unknown	Our laboratory
950661	G	Sputum	Our laboratory
950883	H	Bronchial wash	Our laboratory
941206	I	Sputum	M. Kubin*
950556	J	Lymph node	Our laboratory
951123	K	Blood†	Our laboratory
951064	L	Gastric aspirate	Our laboratory
930001	M	Gastric aspirate	Our laboratory
950895	N	Bronchial wash*	Our laboratory
950818	O	Sputum	Our laboratory
941212	P	Sputum	M. Kubin
941059	Q	Sputum	Our laboratory
930826	R	Vertebral bone	M. Duffaux-Fauville‡
930789	S	Unknown	Our laboratory

* National Institute of Public Health, Prague, Czech Republic.

† Strain isolated from patient with AIDS.

‡ Institut Pasteur du Brabant, Brussels, Belgium.

Table 2. *Mycobacterium* species used in this study

<i>Mycobacterium</i> species	Strain no.	Source or reference
<i>M. xenopi</i>	930664	Our laboratory
<i>M. bovis</i>	14002	CIPT*
<i>M. smegmatis</i>	14133	CIPT
<i>M. gordonae</i>	14021	CIPT
<i>M. chelonae</i>	14042	CIPT
<i>M. fortuitum</i>	14041	CIPT
<i>M. marinum</i>	14012	CIPT
<i>M. avium</i>	950495	Our laboratory
<i>M. intracellulare</i>	14031	CIPT
<i>M. kansasii</i>	14011	CIPT
<i>M. celatum</i>	931227	W. R. Butler†
<i>M. terrae</i>	14032	CIPT

* CIPT: Collection Institut Pasteur Tuberculose, Institut Pasteur, Paris, France

† National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA, USA.

terrae, *M. celatum*, *M. chelonae*, *M. fortuitum* and *M. kansasii*, were investigated to define the host range of IS1395 (Table 2).

Genomic DNA preparation for molecular cloning and RFLP.

Mycobacterial strains were grown in 5 ml Middlebrook 7H9 broth containing 1 mg D-cycloserine ml⁻¹ and incubated overnight at 37 °C. Cells were heated for 20 min at 80 °C, centrifuged, resuspended in 250 µl of 25% (w/v) sucrose, 50 mM Tris/HCl pH 8.0, 50 mM EDTA containing 500 µg lysozyme ml⁻¹ and incubated overnight. Then 250 µl of a solution consisting of 100 mM Tris/HCl, 1% (w/v) SDS and 400 µg proteinase K ml⁻¹ was added and the sample incubated for 4 h at 55 °C. DNA was extracted with phenol/chloroform, precipitated with ethanol and dissolved in TE (10 mM Tris/HCl, pH 8.0, 1 mM EDTA).

Molecular cloning and library screening. Three micrograms of *M. xenopi* DNA was digested with *Bam*HI and separated on a 0.8% (w/v) agarose gel. Restriction fragments of approximately 1–4 kbp were recovered from the agarose gel by electroelution into sterile water using a dialysis membrane. DNA was purified by phenol/chloroform extraction followed by ethanol precipitation and ligated into the *Bam*HI site of the plasmid pBluescript II KS⁺ (Stratagene).

Ligation mixtures were transformed into *Escherichia coli* DH5α by electroporation (Gene Pulser unit; Bio-Rad). Recombinant colonies were selected on Luria-Bertani solid medium (10 g Bacto-tryptone l⁻¹, 5 g yeast extract l⁻¹, 10 g NaCl l⁻¹, pH 7.5, supplemented with 100 µg ampicillin ml⁻¹) and then transferred onto a nylon filter (N⁺ Hybond; Amersham) by standard techniques (Sambrook *et al.*, 1989).

IS1081 probe was prepared from a *M. bovis* strain as described by Collins *et al.* (1993), labelled with [α-³²P]dCTP by using the Megaprime DNA-labelling kit (Amersham), and hybridized to the filters overnight at 65 °C in Rapid hybridization buffer (Amersham) followed by stringent washes at 65 °C: twice for 10 min each in 2 × SSC (1 × SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate), 0.1% (w/v) SDS; twice for 10 min each in 1 × SSC, 0.1% (w/v) SDS and once for 10 min in 0.1 × SSC, 0.1% (w/v) SDS.

Fig. 1. Nucleotide and amino acid sequences of *M. xenopi* IS1395. Imperfect inverted repeats, designated IR-R and IR-L, are shaded. The targets of the primers XNA and XNB used for IS1395 probe preparation are indicated by arrows above the sequence. The deduced amino acid sequence is shown below the DNA sequence, the asterisk indicates the putative transposase termination codon and a potential ribosome-binding site (RBS) is identified.

Fig. 2. Comparison of insertion sites of *M. bovis* IS1081 and *M. xenopi* IS1395 in the recombinant plasmids pB5 (Collins & Stephens, 1991) and pMP1 respectively (this study). The gaps correspond to the IS elements.

Plasmid pMP1 to be used for DNA sequence analysis was purified using Qiagen mini-columns (Qiagen 'Midi' Kit).

Sequencing and analysis. Sequences of double-stranded plasmid DNA were determined by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977) using the *Taq*

DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems), on a GeneAmp PCR System 9600 (Perkin Elmer), and run on a DNA Analysis system model 373 stretch (Applied Biosystems). The sequencing was performed by using a DNA walking strategy along the 2 kbp pMP1 insert.

Similarities with IS1395 were found in databases by using BLAST (Altschul *et al.*, 1990) and FASTA (Pearson & Lipman, 1988) algorithms. Deduced amino acid sequences from IS1395 and members of the IS256 family were aligned by using CLUSTAL W software (Thompson *et al.*, 1994). A distance matrix was produced by using PROTDIST from PHYLIP 3.5 (J. Felsenstein, University of Washington), according to the method of Dayhoff, and a tree was constructed by the neighbour-joining method.

PCR. Sequence analysis of IS1395 allowed the selection of primers XNA (5' CTG CTG TCG GTG TTC ATC C 3') and XNB (5' GGG AAA AGC GGT GAA CGC CAG C 3') at positions 133–151 and 1008–1029, respectively (Fig. 1). The two primers XNA and XNB were used to generate a 897 bp probe for RFLP analysis and to define the host range of IS1395 in PCR tests performed with target DNAs from different mycobacterial species (Table 2). DNA sources consisted of crude lysates obtained by heating cell suspensions at 100 °C for 30 min. For this purpose, a loopful of mycobacteria was suspended in 100 µl TE containing 1% (v/v) Triton X-100.

Amplification reactions were performed with 50 µl volumes containing 1 × *Taq* polymerase buffer (50 mM Tris/HCl pH 8.3, 50 mM KCl, 0.01%, w/v, gelatin) 1.5 mM MgCl₂, 200 µM each deoxynucleoside triphosphate, 1 µmol primers XNA and XNB, and 2 U *Taq* polymerase (Perkin Elmer) covered with mineral oil. Two microlitres of template DNA prepared as indicated above was added to the mix. The 35 amplification cycles (1 min at 95 °C; 1 min at 65 °C; 1 min at 72 °C) were followed by a 10 min extension at 72 °C in a Perkin Elmer thermocycler. Amplification products were analysed by electrophoresis on a 1.2% (w/v) agarose gel and detected by ethidium bromide staining.

RFLP. The 897 bp probe within IS1395 was prepared as described above, purified from agarose gel according to the GeneClean procedure (Bio101) and labelled with horseradish peroxidase using the ECL system (Amersham).

Two micrograms of mycobacterial DNA was digested with 10 U *Pvu*II in a 30 µl reaction mixture. DNA fragments were resolved by overnight electrophoresis on a 1% (w/v) agarose gel at 1.5 V cm⁻¹ and transferred to a nylon membrane (N⁺ Hybond; Amersham) (Sambrook *et al.*, 1989). The membrane was hybridized overnight at 42 °C with the probe, then washed twice for 10 min at 55 °C with 0.5 × SSC containing 0.4% (w/v) SDS, and finally washed twice for 5 min at room temperature with 2 × SSC. The peroxidase-labelled probe was detected with the ECL detection system (Amersham).

Host range investigation. Mycobacterial DNAs from different species (Table 2) were digested with *Pvu*II, transferred to a nylon membrane and probed with the 897 bp fragment within IS1395 labelled with ³²P as described above (Fig. 4).

PFGE. *M. xenopi* isolates were cultivated in 5 ml Middlebrook 7H9 broth. Cultures were inoculated into 40 ml fresh medium at an OD₆₅₀ of 0.08, and incubated at 42 °C till an OD₆₅₀ of 0.2 was obtained. Cells were centrifuged, resuspended in 1% (v/v) Triton X-100 and incubated for 2 h at 37 °C. They were then centrifuged again, washed in 0.05 M EDTA and resuspended in 10 mM Tris/HCl pH 8.0, containing 100 mM EDTA and 1 mg lysozyme ml⁻¹. The suspension was then mixed with an equal

Fig. 3. Unrooted phylogenetic tree of insertion sequence transposases related to *M. xenopi* IS1395 calculated by the neighbour-joining method (NJ). Percentage amino acid similarities between IS1395 and the other elements are indicated in parentheses.

volume of 1% (w/v) low-melting-point agarose and dispensed in a slot former of 60 μ l. Plugs were incubated in 0.5 M EDTA for 24 h at 37 °C, then washed in TE and incubated in 1% (w/v) sodium *N*-lauroylsarcosinate (Sigma) and 2 mg proteinase K ml^{-1} for an extra night at 55 °C. Plugs were washed in TE and incubated in TE containing 4 μ g PMSF ml^{-1} (Sigma) for 1 h at 55 °C to inactivate proteinase K. Finally, plugs were extensively washed in TE and digested with 30 U *Dra*I. After digestion, plugs were loaded onto a 0.8% (w/v) agarose gel. Large restriction fragments were separated by zero-integrated-field electrophoresis (ZIFE) with the AutoBase system (Q-Life Systems) for 65 h at room temperature with the 8–200 kb ROM card.

RESULTS

Description of IS1395

In this study, we produced an *M. xenopi* partial library of *Bam*HI-digested genomic DNA and screened it using an IS1081 probe prepared from *M. bovis* as described by Collins *et al.* (1993). One recombinant clone gave a strong hybridization signal suggesting the presence of the IS1081-like element. Further analysis of this clone revealed that it contained a plasmid, named pMP1, harbouring a DNA insert of approximately 2 kbp.

DNA sequencing of the pMP1 insert revealed the

characteristic features of an IS. This element, designated IS1395, is 1323 bp long and contains at its extremities 15 bp inverted repeats (IR) with one mismatch (Fig. 1). The base composition is typical of mycobacteria, with a 65.2 mol% G+C content. Analysis of the sequence showed the presence of a large ORF which could encode a protein of 415 amino acids with ATG as a putative start codon at position 52 and preceded by a potential ribosome-binding site at the expected position (Fig. 1). The transposases of the other IS256 mycobacterial elements (described below) have the same start codon.

The GenBank database was screened to identify elements related to IS1395. The nucleotide sequence showed 86% homology to *M. bovis* IS1081, and less significant homologies to *M. avium* IS1311/IS1245 and *Pseudomonas cepacia* IS406. Moreover, comparison of the IS1395 to the *M. bovis*/*M. bovis* BCG IS1081 flanking regions revealed 86% identity in a 39 bp overlap with the 'right arm' and 79% identity in a 91 bp overlap with the 'left arm' (Fig. 2). These flanking regions did not present peculiar base composition or direct repeats (DR).

When protein databases were screened for elements homologous to the putative protein encoded by the large ORF, significant homologies were found with putative transposases belonging to the *Staphylococcus aureus* IS256

Fig. 4. Southern blot analysis of *PvuII*-digested DNAs of mycobacterial species probed with IS1395. The molecular mass marker is bacteriophage λ DNA restricted by *PstI*. The weak bands indicated by arrows are better visualized after a longer exposure.

family. Amino acid sequence comparisons revealed the highest scores for transposases from *M. bovis* IS1081, *M. avium* IS1245 and IS1311, *Rhizobium meliloti* ISRm3, *P. cepacia* IS406 and *S. aureus* IS256. A phylogenetic tree based on amino acid sequence similarities was constructed by the neighbour-joining method (Fig. 3).

IS1395 host range

The occurrence of IS1395 among members of the genus *Mycobacterium* was investigated by hybridization of digested DNA from different mycobacterial species with IS1395. Patterns consisting of several bands were displayed by DNA from *M. bovis*, *M. goodii*, *M. avium* and *M. celatum* whereas no hybridization was detected for DNA from the other species examined (Fig. 4). Identical patterns were obtained for *M. bovis* and *M. avium* when using IS1081 and IS1245/IS1311 as respective probes, confirming that hybridization occurred with these IS1395-related elements. Moreover PCR tests performed with primers XNA and XNB allowed for the amplification of the expected 897 bp fragment within IS1395 in DNA templates from *M. xenopi* only and did not yield any amplified products with DNA templates from the other mycobacterial species listed in Table 2 (data not shown).

Distribution of IS1395 within *M. xenopi* strains

PFGE of *M. xenopi* non-digested genomic DNA revealed an extra-chromosomal element in all strains (Fig. 5a). Using different electrophoresis conditions with changes in pulse duration, the mobility of the extra-chromosomal DNA was consistent with a linear fragment with a molecular mass in the region of 150 kbp. PFGE was performed in the presence or the absence of the intercalating agent ethidium bromide in order to distinguish between linear and supercoiled circular DNA (data not shown). The estimated size of the extra-chromosomal element was unaffected by the presence of ethidium bromide. These findings were consistent with linearity of the element (Ferdows *et al.*, 1996). To our knowledge, this is the first linear extra-chromosomal element ever described in mycobacteria.

Following PFGE, DNA was blotted and probed with IS1395. Hybridizing bands were visualized on the chromosomes of all the strains. An additional signal was observed on the extra-chromosomal element in one instance as shown in Fig. 5, lane 8. Because of PFGE conditions adapted to the resolution of large DNA fragments, the molecular mass of the extra-chromosomal element harbouring the IS could not be differentiated from that of the extra-chromosomal element devoid of IS1395.

To study the RFLP of the repetitive element among *M. xenopi* strains, primers XNA and XNB were designed to produce an IS1395 internal probe. *PvuII* was used for genome digestion as IS1395 did not contain a corresponding restriction site.

Analysis of the Southern blot of *PvuII*-digested DNAs of 19 non-related strains, i.e. clinical strains from different patients (Table 1), showed that IS1395 was present in multiple copies in the *M. xenopi* genome and generated strain-specific patterns. The copy number varied from 3 to 18 (median 14) (Fig. 6). Nevertheless, the DNA fingerprints observed were not highly polymorphic: the RFLP patterns displayed several bands in common. In the region of 2.5 kbp, 15 out of the 19 studied strains showed either five or two bands (Fig. 6a). Stability of the RFLP patterns was demonstrated by typing *M. xenopi* strains recovered from hospital tap water from July 1993 through June 1995. The 23 month interval did not lead to profile changes (data not shown). Therefore, despite its potential ability to transpose, IS1395 is stable enough to be considered as an epidemiological marker useful for strain typing.

RFLP and PFGE analysis were conducted on the same strains to compare the epidemiological value of these techniques (Fig. 6). The PFGE patterns consisted of about 25 large restriction fragments and, like RFLP profiles, presented bands shared by all strains. In our hands, the 200–300 kbp range gave the best resolution and corresponded to the most polymorphic region. The two techniques afforded similar discrimination between strains. However RFLP could provide a better differentiation as observed in lanes G and H: the PFGE profiles

Fig. 5. (a) PFGE separation of undigested genome of *M. xenopi* strains. Lanes 1 to 9 correspond to strains isolated from patients I, M, O, F, D, K, E, Q and N, respectively. The molecular mass marker consists of DNA concatemers of the bacteriophage λ genome. (b) PFGE probed with IS1395. The molecular mass marker consists of DNA concatemers of the bacteriophage λ genome.

were identical whereas the RFLP patterns differed by three bands.

DISCUSSION

Previous studies showed that *M. xenopi* contained an element related to IS1081, one of the repetitive DNA sequences specific for the tubercle bacilli (Collins *et al.*, 1993; Collins, 1994). In the present study, IS1081 was used as a probe for screening an *M. xenopi* partial genomic library. This attempt led to the isolation of a new repetitive element, designated IS1395, which occurs in all *M. xenopi* strains tested. The entire 1323 bp nucleotide sequence was determined and revealed imperfect 15 bp terminal IR. A large ORF, which encodes a putative transposase of 415 amino acids, covers most of IS1395. The putative protein sequences of the different IS256 family members showed that mycobacterial elements belong to the two IS256 subclasses: one subclass comprises IS1081, specific for tubercle bacilli (van Soolingen *et al.*, 1992), IS1245 and IS1311, specific for *M. avium* (Guerrero *et al.*, 1995; Roiz *et al.*, 1995) and IS1395, whereas IS6120 (from *M. smegmatis*) belongs to the other IS256 subclass, characterized by a large ORF preceded by a smaller one (Guilhot *et al.*, 1992).

The IS1395 and IS1081 left and right flanking sequences displayed a high percentage of identity over 130 nucleotides (Fig. 2), whereas homology no other sequence in the databases could be detected. A recent study showed that IS1081 cloned from *M. bovis* BCG exhibited the same flanking sequence as that of IS1081 previously isolated from a *M. bovis* strain (Collins & Stephens, 1991). Moreover, the sequence of the 5' end of an IS1395 copy harboured by a *Pst*I restriction fragment showed an identical flanking sequence (data not shown). These results indicate that IS1081 and IS1395 are inserted at specific identical positions on the chromosome, accounting for the limited polymorphism generated by IS1081 in tubercle bacilli. However, although RFLP shows that most *M. xenopi* strains have several IS1395 copies in the same chromosomal loci, IS1395 provides useful poly-

morphism for *M. xenopi* strain typing, based on variations of both copy number and distribution within the genome. This suggests that IS1081 has a lower degree of mobility or a higher degree of insertion specificity than IS1395. An alternative explanation is that *M. bovis* as a species is less variable than *M. xenopi*, and that the factors responsible for the greater homogeneity of *M. bovis* also result in less polymorphism of the IS. This latter explanation is also supported by the lack of polymorphism found in *M. bovis* when considering IS6110 RFLP patterns (van Soolingen *et al.*, 1992).

Whereas transposition events usually generate duplication of a few nucleotides of the target site (Galas & Chandler, 1989), no DR could be detected in the IS1081 or IS1395 flanking sequences.

The high percentage homology between the IS1395 from *M. xenopi* and IS1081 from *M. bovis* may indicate that the element existed before the divergence of the organisms or that a horizontal transfer occurred later after the divergence. Since the species which host IS256-related elements are taxonomically remote, a horizontal gene transfer may be hypothesized. It has been demonstrated in different Gram-positive species that IS256 and related ISs can coexist in multiple copies in a cell on both plasmids and chromosome (Wheatford & Laberge, 1991; Hesselbarth *et al.*, 1995). In the present study, we characterized a large extra-chromosomal element in *M. xenopi* which, in one strain out of the nine tested, harboured IS1395, thus providing a plausible mechanism for IS transfer. In *M. avium*, plasmids of various molecular mass have been found (Meissner & Falkinham, 1986), but comparative analyses of IS distributions and plasmid profiles are lacking.

IS1395-related elements were detected in *M. goodii* and *M. celatum*, a recently described opportunistic agent (Butler *et al.*, 1993; Tortoli *et al.*, 1995). The characterization of these related IS256 elements present in the two species will provide valuable information on the genetic relatedness and evolution of these mycobacteria. On the basis of 16S rRNA homology and phenotypic

Fig. 6. (a) RFLP patterns of *M. xenopi* DNAs probed with IS1395. The molecular mass marker is bacteriophage λ DNA restricted by *Pst*I. (b) PFGE patterns of *M. xenopi* DNA digested with *Dna*I. The molecular mass marker consists of DNA concatemers of bacteriophage λ genome. Lane designations correspond to the patient identifiers in Table 1.

biochemical tests, *M. xenopi*, *M. avium* and *M. celatum* are related species, whereas the *M. tuberculosis* complex or *M. gordonae* belong to distant taxonomic groups (Butler *et al.*, 1993; Rogall *et al.*, 1990). As indicated by Wayne *et al.*

(1996), in a semantide-based analysis of slowly growing mycobacteria, the attempt to base phylogenetic schemes on 16S rRNA alone has not been confirmed, partly because the depth of branching in the tree somehow

reflects the age of the branch. Very recently diverged species may not be distinguishable by 16S rRNA sequencing (Fox *et al.*, 1992). Trees constructed using other, independent semantides, e.g. DNA sequences of conserved genes (Kapur *et al.*, 1995; Zolg & Philipp-Schulz, 1994) or ISs, probably represent various molecular clocks. Analyses based on such diverse markers may contribute to shedding light on mycobacterial taxa not well resolved by 16S rRNA analysis and/or reveal some unexpected relatedness suggesting common ancestors for otherwise remote organisms.

As previously shown by Collins (1994), IS1081 may be used as an epidemiological marker to differentiate *M. xenopi* strains. Our study confirms these results, and RFLP patterns were found to be identical using IS1081 or IS1395 as probes (data not shown). RFLP analysis allowed to separate *M. xenopi* strains into two major 'sub-groups' according to the presence of five or two bands in the 2.5 kbp region. Strain differentiation was comparable by RFLP and by PFGE. PFGE patterns within each RFLP 'sub-group' were more homogeneous than patterns from different 'sub-groups'. As RFLP upon hybridization with IS1395 and PFGE after *Dra*I digestion are two independent techniques (IS1395 has no *Dra*I restriction site), which rely on the polymorphism of distinct DNA sequences, similarities observed between profiles with both methods stress the high homogeneity of the *M. xenopi* genome. Some strains indistinguishable by PFGE displayed distinct RFLP patterns. However, the demonstration of better performance for RFLP has to be established on a larger set of *M. xenopi* strains.

In conclusion, DNA fingerprinting with the IS1395 probe represents a potentially useful tool for epidemiological studies. Further epidemiological studies to evaluate this repetitive element are under way, especially to determine possible relationships between RFLP patterns and *M. xenopi* sources (clinical isolates, environment, animals etc.). Moreover, the characterization of the molecular properties of the IS1395-related elements in *M. goodii* and *M. celatum* will contribute to a better knowledge of mycobacterial ISs, their mode of transposition and dissemination.

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