ELSEVIER

FEMS Microbiology Letters 245 (2005) 249–255

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Mycoplasma bovis shares insertion sequences with *Mycoplasma agalactiae* and *Mycoplasma mycoides* subsp. *mycoides* SC: Evolutionary and developmental aspects

Anne Thomas [a], Annick Linden [b], Jacques Mainil [b], Daniela F. Bischof [c], Joachim Frey [c], Edy M. Vilei [c,*]

[a] *Department of Morphology and Pathology, University of Liège, B43A, Sart Tilman, 4000 Liège, Belgium*
[b] *Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, B43A, Sart Tilman, 4000 Liège, Belgium*
[c] *Institute of Veterinary Bacteriology, University of Bern, Länggass-Strasse 122, Postfach, 3001 Bern, Switzerland*

Received 5 November 2004; received in revised form 3 January 2005; accepted 7 March 2005

First published online 14 March 2005

Edited by W. Schumann

Abstract

Three new insertion elements, IS*Mbov1*, IS*Mbov2* and IS*Mbov3*, which are closely related to IS*Mag1* (*Mycoplasma agalactiae*), IS*Mmy1* and IS*1634* (both *Mycoplasma mycoides* subsp. *mycoides* SC), respectively, have been discovered in *Mycoplasma bovis*, an important pathogen of cattle. Southern blotting showed that the genome of *M. bovis* harbours 6–12 copies of IS*Mbov1*, 11–15 copies of IS*Mbov2* and 4–10 copies of IS*Mbov3*, depending on the strain. A fourth insertion element, the IS*30*-like element, is present in 4–8 copies. This high number of IS elements in *M. bovis*, which represent a substantial part of its genome, and their relatedness with IS elements of both *M. agalactiae* and *M. mycoides* subsp. *mycoides* SC suggest the occurrence of two evolutionary events: (i) a divergent evolution into *M. agalactiae* and *M. bovis* upon infection of different hosts; (ii) a horizontal transfer of IS elements during co-infection with *M. mycoides* subsp. *mycoides* SC and *M. bovis* of a same bovine host.

Keywords: *Mycoplasma bovis*; Insertion sequences; Southern blotting; Distribution; Sequence analysis

1. Introduction

Mycoplasma bovis is the most important mycoplasma species in cattle in countries free of contagious bovine pleuropneumonia (CBPP). It is widespread in North America [1,2] and in Europe where it is associated with bronchopneumonia and arthritis in calves, and with mastitis and genital infections in adult cattle [3–7].

A large number of insertion sequences (IS) has been described in mollicutes [8–13] and are useful genetic markers for diagnosis and epidemiological analysis. IS elements are mobile DNA fragments (<2.5 kb), often present in multiple copies and causing a significant degree of plasticity of prokaryotic genomes, thus leading to the appearance of variants and subtypes of bacterial species.

M. bovis is characterized by its antigenic variation associated with DNA-recombinations [14]. Recent studies [15] have emphasized the presence of recombinase genes in several mycoplasmas, including *M. bovis*.

* Corresponding author. Tel.: +41 31 631 2369; fax: +41 31 631 2634.
E-mail address: edy.vilei@vbi.unibe.ch (E.M. Vilei).

0378-1097/$22.00
doi:10.1016/j.femsle.2005.03.013

Moreover, a gene encoding a putative IS*30*-like protein in *M. bovis* type strain PG45 was identified [16]. In addition, *Mycoplasma agalactiae*, which is phylogenetically closely related to *M. bovis*, presents an IS element, IS*Mag1*, whose probe reacted with DNA of *M. bovis* [13]. Interestingly, the insertion sequence IS*Mmy1* identified recently in *Mycoplasma mycoides* subsp. *mycoides* small colony (SC) type seemed to be present also in *M. bovis* [12]. *M. mycoides* subsp. *mycoides* SC harbours indeed two additional IS elements, IS*1296* [9] and IS*1634* [11], that were referred to be absent in *M. bovis*. Thus, the presence of multiple, heterologous IS elements in the genomes of pathogenic mycoplasma species is not unusual.

The present study reports the presence of three IS elements in *M. bovis*, IS*Mbov1*, IS*Mbov2* and IS*Mbov3*, which are phylogenetically related to IS*Mag1*, IS*Mmy1* and IS*1634*, respectively, and shows the distribution of these three plus a further IS element (IS*30*-like) [16] in eleven *M. bovis* isolates differing in pathogenic and cultural features.

2. Materials and methods

2.1. Mycoplasma strains, growth conditions and DNA extraction

The mycoplasma strains used in this study are listed in the Table 1, according to their origin, their pathological and cultural features. All *M. bovis* strains were grown in modified Hayflick broth medium during 24–48 h [17]. Mycoplasma strains 2610, 0435 and 9585 were also subcultured several times (116, 80 and 98 times, respectively), through liquid medium at intervals of 48 h. Cells were harvested by centrifugation at 8000×*g* for 15 min, washed with phosphate-buffered saline (PBS) solution (140 mM NaCl, 2.7 mM KCl, 15 mM KH_2PO_4, 8 mM Na_2HPO_4, pH 7.4) and re-suspended in PBS. Mycoplasmal genomic DNA was extracted by phenol/chloroform method as described previously [18]. DNA concentration was determined spectrophotometrically with GeneQuantI (Amersham Pharmacia Biotech). All *M. bovis* strains tested were confirmed to belong to this species by sandwich ELISA [17] and the *uvrC* specific PCR [19].

2.2. PCR amplification and preparation of DNA probes for Southern blotting

Polymerase chain reaction (PCR) was performed in a DNA thermal cycler Gene Amp 9600 (Applied Biosystems) in a 50-μl reaction mixture [50-mM Tris–HCl, pH 9.2, 1.75 mM $MgCl_2$, 16 mM $(NH_4)_2SO_4$, 350 μM of each dNTP] which contained approximately 50 ng of genomic template DNA, 300 nM of each primer, and 1.75 U of a mixture of *Taq* and *Pwo* DNA polymerases (Expand Long Template PCR System kit, Roche Diagnostics). The mixtures were subjected to 2 min denaturation at 94 °C followed by 30 cycles of amplification with the parameters: 30 s at 94 °C, 30 s at 48 °C, and 2 min extension at 68 °C. Digoxigenin-11-dUTP (DIG)-labelled probes were produced by PCR as described above in the presence of 50 μM DIG (Roche Diagnostics).

The IS*Mag1* specific probe was prepared with primers Maga-IS-L and Maga-IS-R [13], using DNA from *M. agalactiae* strain 3990. The IS*Mbov1* specific probe was constructed using the oligonucleotide primers MBOV-IS-L and MBOV-IS-R (Table 2), and DNA

Table 1
M. bovis strains used in this study

Strain designation	Passage number	Country of origin	Source[a]	Disease	Adherence rates (%) to EBL cells[b]
PG45[c]	Unknown[d]	USA	Milk	Mastitis	5.0
ML1[e]	7	France	Lung	Bronchopneumonia	6.4
221/89	7	Germany	Milk	Without symptoms	5.9
86p	7	Belgium	Milk	Mastitis	11.3
39G	7	Belgium	BAL	Bronchopneumonia	13.8
2610	7	UK	Joint fluid	Arthritis	14.7
2610	116	UK	Joint fluid	Arthritis	2.9
0435	7	Belgium	BAL	Bronchopneumonia	15.7
0435	80	Belgium	BAL	Bronchopneumonia	ND[f]
9585	7	Belgium	BAL	Bronchopneumonia	13.6
9585	98	Belgium	BAL	Bronchopneumonia	8.0

[a] BAL, bronchoalveolar lavage.
[b] EBL, embryonic bovine lung. See Ref. [31].
[c] PG45, type strain of *M. bovis*.
[d] The passage number is however >15.
[e] ML1, rabbit isolate; all other strains are of bovine origin.
[f] ND, not determined.

Table 2
Oligonucleotide primers used

Primer	Sequence (5′–3′)	Melting temperature (°C)[a]
MBO-ISYOG-L	GAATTATACTAAAAATTATAATCATC	46
MBO-ISYOG-R	GACACCATAGTCATTAGCTAAC	52
ISMBO-L	TGTCCATTAAATACATTAAATTATC	48
ISMBO-R	TATATTTTTAAAATAGACTTCAATTC	48
MBOV-IS-L	CATTAACAAAGCAAAAAGCACC	53
MBOV-IS-R	AAAGCACCTAATTTGAGTATTG	51
IS1634(in)L2	GAAATTTAAATGCAAAAATTTGTGC	52
IS1634(in)R2	TTTTGAATTAAAATGTCTCTATCG	51

[a] Obtained with the "Oligonucleotide Properties Calculator" at the website www.basic.nwu.edu/biotools/oligocalc.html, using the nearest neighbour method and the parameters 300 nM primer and 50 mM salt (Na^+).

from *M. bovis* strain PG45. The IS*Mmy1* specific probe was prepared with primers 5im_ismmy1 and 3im_ismmy1 [12], using DNA from *M. mycoides* subsp. *mycoides* SC strain PG1. The IS*Mbov2* specific probe was constructed using the oligonucleotide primers ISMBO-L and ISMBO-R (Table 2), and DNA from *M. bovis* strain PG45. The IS*1634* specific probe was prepared with primers IS1634(in)L and IS1634(in)R [11], using DNA from *M. mycoides* subsp. *mycoides* SC strain PG1. The IS*Mbov3* specific probe was constructed using the oligonucleotide primers IS1634(in)L2 and IS1634(in)R2 (Table 2), and DNA from *M. bovis* strain PG45. The IS*30*-like specific probe was constructed using the oligonucleotide primers MBO-ISYOG-L and MBO-ISYOG-R (Table 2), and DNA from *M. bovis* strain PG45.

2.3. Identification and isolation of ISMbov1, ISMbov2 and ISMbov3

A genomic library was obtained from the isolate 2610 (passage 7) (Table 1). Genomic *Sau*3AI fragments of sizes between 1.0 and 8.0 kb were cloned into the *Bam*HI site of pBluescriptII SK+ (Stratagene). Ligation products were transformed into XL1-Blue MRF′ *Escherichia coli* (Stratagene) and transformants were grown on LB agar plates containing ampicillin (50 μg/ml), X-gal (80 μg/ml) and IPTG (20 mM). Colony hybridization at 56 °C was performed with the genomic library previously transferred onto Whatman paper filters (540, VWR International) using the probes for IS*Mag1*, IS*Mmy1* and IS*1634* following the standard protocol [20]. Plasmid DNA of the selected positive colonies was isolated using the Plasmid Midi kit (Qiagen AG). The clones obtained by IS*Mag1* hybridization harboured the *M. bovis* insertion element designated IS*Mbov1*, those obtained by IS*Mmy1* hybridization harboured the insertion element designated IS*Mbov2* and those obtained by IS*1634* hybridization harboured the insertion element designated IS*Mbov3*.

2.4. Sequence analysis of the IS genes

DNA sequencing was performed with a DNA Sequenator AB 3100 and the *Taq* Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems). In the first step, oligonucleotide primers containing the T3 and T7 promoter sequences flanking the cloning site of the pBluescriptII SK+ vector were used. The sequences were completed by "primer walking" using synthesized oligonucleotides. For the analysis of the complete cloned segments, the deletion technique was employed by using exonuclease III of the double-stranded Nested Deletion kit (Amersham Pharmacia Biotech). The DNA sequences were assembled using the Sequencher 3.0 (GeneCodes) software. Alignments were done with PILEUP and sequence comparisons with FASTA and BESTFIT from the GCG Wisconsin package (Genetics Computer Group, Inc., Madison, WI). The deduced amino acid sequences were analysed with the program PROSITE [21].

2.5. Nucleotide sequence Accession Numbers

The EMBL/GenBank Accession Nos. for the nucleotide sequences of one representative copy of each insertion element of *M. bovis* determined in this work are: AJ564386 for IS*Mbov1*, AJ536157 for IS*Mbov2* and AJ829923 for IS*Mbov3*.

2.6. Southern blotting

Genomic mycoplasmal DNA was digested by *Eco*RV, a restriction enzyme not cutting within the sequences used as probes, submitted to electrophoresis on 0.7% (w/v) agarose gels and transferred onto positively charged nylon membranes (Roche Diagnostics) following standard protocol [20]. The membranes were pre-incubated with 10 ml hybridization buffer [5 X SSC (1 X SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.7), 0.1% *N*-lauroylsarcosine, 0.02% SDS and 1%

(w/v) blocking reagent (Roche Diagnostics)] per 100 cm^2 membrane at 68 °C for 2 h and then hybridized over night at 68 °C with 5 ml hybridization buffer containing 1 μg DIG-labelled probes (for IS*30*-like, IS*Mbov1*, IS*Mbov2* and IS*Mbov3*) per 100 cm^2 membrane. The membranes were washed twice for 5 min at room temperature with 2 X SSC containing 0.1% SDS, and twice for 15 min at room temperature with 0.2 X SSC containing 0.1% SDS. The DIG-labelled probes were detected using phosphatase-labelled anti-digoxigenin antibodies and CDP-*Star* (Roche Diagnostics) according to the manufacturer's instructions.

3. Results

3.1. Characterization of *IS*Mbov1

While reacting a genomic library from *M. bovis* with the IS*Mag1* specific probe derived from the DNA of the ovine mycoplasma *M. agalactiae*, a new insertion element was discovered in the bovine pathogen. This IS element of 1521 bp, named IS*Mbov1*, shows high homology (92%) with IS*Mag1* and contains inverted repeats of 3 bp. The encoded transposase (nucleotides 333–1361) is composed of 342 amino acids (Fig. 1(a)). An integrase core domain (Pfam accession number PF00665) was localized between bases 192 and 339.

3.2. Characterization of *IS*Mbov2

From the above library, a positive clone reacting with a gene probe derived from IS*Mmy1* of *M. mycoides* subsp. *mycoides* SC was retained and analyzed in detail. A full IS element of 1671 bp was evidenced, named IS*Mbov2*, that shows a very high homology (97%) with IS*Mmy1* and contains inverted repeats of 30 bp and a gene encoding the putative transposase on a single ORF (Fig. 1(b)). The encoded transposase (nucleotides 235–1647) is composed of 470 amino acids. A transposase DDE domain (Pfam accession number PF01609) [22] was detected between amino acids 172 and 385.

3.3. Characterization of *IS*Mbov3

The *M. bovis* genomic library reacted also with the IS*1634* specific probe. Sequencing of a positive clone revealed the presence of an IS element of 1873 bp, named IS*Mbov3*, that shows 97% identity with IS*1634* of *M. mycoides* subsp. *mycoides* SC and contains inverted repeats of 13 bp and a gene encoding a transposase (nucleotides 184–1785) of 533 amino acids (Fig. 1(c)). A conserved integrase C1 signature sequence of IS*4* family transposases was detected in the C-terminal half of the transposase between amino acids 416 and 430.

3.4. Distribution of *IS*30-like, *IS*Mbov1, *IS*Mbov2 and *IS*Mbov3 in M. bovis strains

Genomic DNA from 11 *M. bovis* isolates (Table 1), strain PG1 of *M. mycoides* subsp. *mycoides* SC and strain 3990 of *M. agalactiae* digested by *Eco*RV was subjected to Southern blotting with probes for the IS elements IS*30*-like, IS*Mbov1*, IS*Mbov2* and IS*Mbov3*. The four typing experiments led to the identification of different hybridization patterns with heterogeneous profiles. Among the 11 *M. bovis* isolates, the band patterns revealed the presence of different copy numbers of the four IS elements.

IS*30*-like typing evidenced the presence of 4–8 copies, depending on the strain (Fig. 2). PG45 had a particular profile, whereas the other *M. bovis* strains formed three similarity clusters. The IS*30*-like specific probe also reacted with an *Eco*RV DNA fragment from *M. agalactiae* strain 3990 (Fig. 2). No pattern similarity was observed among the 11 *M. bovis* isolates by IS*Mbov1*

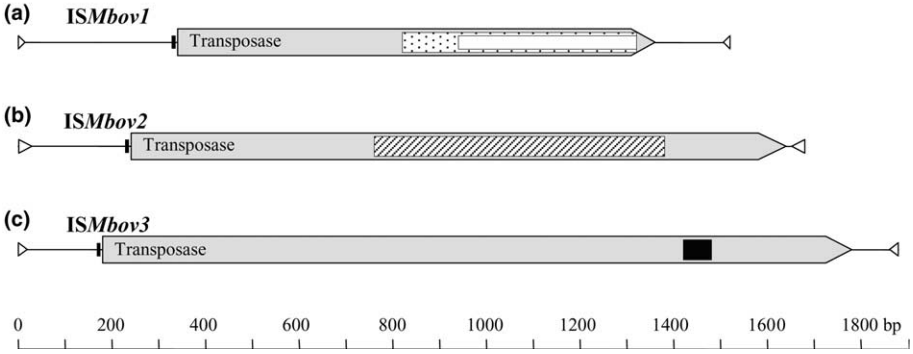

Fig. 1. Structures of IS*Mbov1*, IS*Mbov2* and IS*Mbov3*. The genetic maps for IS*Mbov1* (panel a), IS*Mbov2* (panel b) and IS*Mbov3* (panel c) show inverted repeats at the sites of insertion, represented as white triangles. The open reading frames coding for the three transposases are shown as large grey arrows and are preceded by ribosome binding sites, denoted as vertical black bars. The dotted box represents the IS*30* family domain and the white box represents the integrase core domain in the IS*Mbov1* transposase (a). The hatched box indicates the transposase DDE domain in IS*Mbov2* (b). The black box indicates the conserved integrase C1 signature region of IS*4* family transposases in IS*Mbov3* (c).

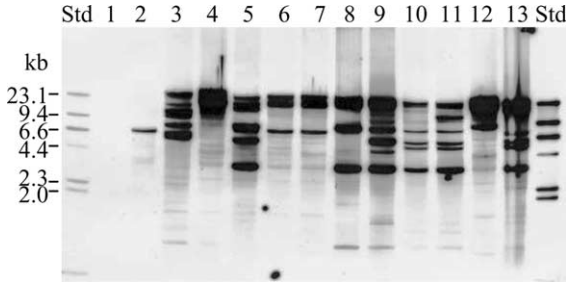

Fig. 2. Detection of the IS*30*-like element. Southern blots of genomic mycoplasma DNA cut with *Eco*RV were probed with a DIG-labelled IS*30*-like probe. The strains used were: (1) PG1, type strain of *M. mycoides* subsp. *mycoides* SC; (2) 3990 of *Mycoplasma agalactiae*; (3) PG45, type strain of *M. bovis*; and the field strains of *M. bovis*; (4) ML1, isolated from rabbit; (5) 221/89; (6) 86p; (7) 39G; (8) 2610 passage 7; (9) 2610 passage 116; (10) 0435 passage 7; (11) 0435 passage 80; (12) 9585 passage 98; (13) 9585 passage 7.

typing (Fig. 3). The number of fragments reacting with the IS*Mbov1* specific probe varied from 8 to 12. Due to the high similarity between IS*Mag1* and IS*Mbov1*, also the *M. agalactiae* strain 3990 presented several bands reacting with the IS*Mbov1* specific probe (Fig. 3). Twelve to 18 DNA fragments from the *M. bovis* strains tested reacted with the IS*Mbov2* specific probe (Fig. 4). Seven isolates presented unique profiles. Strains 86p and 39G presented a same band pattern. Both isolates of strain 0435 (passage 7 and passage 80) presented a similar hybridization profile with few differences. As

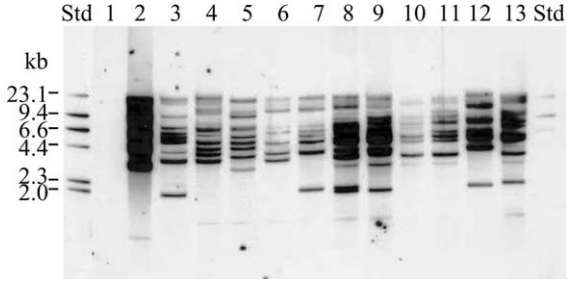

Fig. 3. Detection of IS*Mbov1*. Southern blots of genomic mycoplasma DNA cut with *Eco*RV were probed with a DIG-labelled IS*Mbov1* probe. The strains used were the same as in Fig. 2.

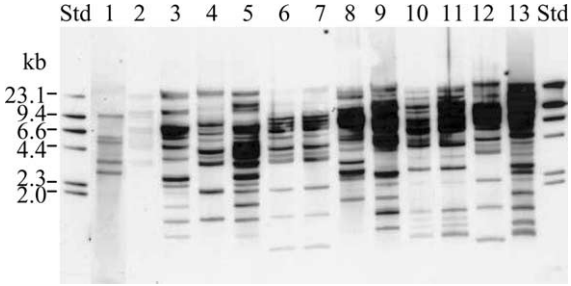

Fig. 4. Detection of IS*Mbov2*. Southern blots of genomic mycoplasma DNA cut with *Eco*RV were probed with a DIG-labelled IS*Mbov2* probe. The strains used were the same as in Fig. 2.

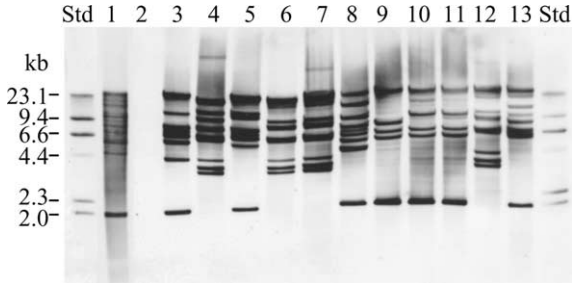

Fig. 5. Detection of IS*Mbov3*. Southern blots of genomic mycoplasma DNA cut with *Eco*RV were probed with a DIG-labelled IS*Mbov3* probe. The strains used were the same as in Fig. 2.

expected, the *M. mycoides* subsp. *mycoides* SC strain PG1 reacted with the IS*Mbov2* specific probe. The bands observed were five (Fig. 4). On the contrary, the IS*Mbov2* specific probe did not react with the *M. agalactiae* strain 3990. IS*Mbov3* typing evidenced the presence of 4–10 copies in *M. bovis*, depending on the strain (Fig. 5). Six isolates presented unique profiles. Strains 86p and 39G and isolate 9585 (passage 98) presented a same *Eco*RV band pattern, as it was the case also between the two isolates of strain 0435 (passage 7 and passage 80). Due to the very high similarity between IS*1634* and IS*Mbov3*, the *M. mycoides* subsp. *mycoides* SC strain PG1 also reacted with the IS*Mbov3* specific probe.

4. Discussion

Three IS elements, IS*Mbov1*, IS*Mbov2* and IS*Mbov3*, have been sequenced from *M. bovis*. Different copies of all three IS elements were sequenced from isolate 2610 (passage 7) and shown to be well conserved at the nucleotide sequence level. Minor variations were observed, with a range of 0.5–11.25 differences per 1000 bp, and only half of them are able to affect the amino acid composition of the three putative transposases. Based on the criteria adopted by Mahillon and Chandler [23] review on IS elements, IS*Mag1* and IS*Mbov1* may be considered isoforms (less than 10% nucleotide divergence). The relationships between IS*Mmy1* and IS*Mbov2*, and between IS*1634* and IS*Mbov3* are even stricter. All the *M. bovis* strains tested in this study contained several copies of each IS. It has been previously described that *M. bovis* contained a further IS element designated IS*30*-like [16]. This IS element is present in all the *M. bovis* strains tested in this study and Southern blotting profiles were not the same as those obtained for IS*Mbov1*, ISM*bov2* and IS*Mbov3*. No correlation has been found between IS profiles and the clinical symptoms of the animals from which the *M. bovis* strains were isolated (bronchopneumonia, arthritis, mastitis, cattle, rabbit), the number of passages, or adherence rates of the strains (Table 1). However, it is evident that the genome of

M. bovis contains a large number of IS elements and this feature could be associated with a rapid gene rearrangement, as observed for *Mycoplasma fermentans* [24].

A clear picture can be observed from the different Southern blot analyses, where the frequency of IS elements and the variation in IS profiles in *M. bovis* contrast, for instance, with the situation in *M. mycoides* subsp. *mycoides* SC, where the profiles for the three different IS elements are rather homogeneous among the strains [11,12,25]. Additionally, in *M. bovis* strains 2610, 0435 and 9585 subjected to more than 80 passages in vitro, further transposition or recombination events could be detected on the Southern blots reacted with the probes for all four IS elements, if compared to the strains after only 7 passages. This indicates that genetic variants of *M. bovis* may arise upon extended growth in vitro. An in vitro passaging effect was also observed in *M. fermentans*, whereby analysis of insertion element sequences revealed inter- and intra-strain polymorphisms [26].

The presence of IS*Mbov1* (homologous to IS*Mag1*) and of IS*30*-like in *M. bovis* and *M. agalactiae* indicates horizontal gene transfer between the two species or suggests that both mycoplasmas had a common ancestor. Note however, that the *M. agalactiae* strain 3990 shows only a single copy of IS*30*-like, while it shows about 25 copies of the IS*Mbov1*-homologue IS*Mag1*. The association of *M. bovis* and *M. agalactiae* was also underlined by the previous findings that both mycoplasmas show a very close phylogenetic relationship, as shown by 16S rRNA and *uvr*C sequence similarities [19,27,28]. In this respect, it is interesting to note that IS*Mbov1* and IS*Mag1* are more closely related to each other than are the housekeeping genes *uvrC* from *M. bovis* and *M. agalactiae* (83% identity at the nucleic acid level) [19]. Previous experiments based on *uvrC* analysis [29] suggested that *M. bovis* might have evolved more recently than *M. agalactiae*. Data as to whether *M. agalactiae* and *M. bovis* have ever been isolated from a common host are, however, not available.

M. bovis can be differentiated from *M. agalactiae* by the presence of multiple copies of IS*Mbov2* and IS*Mbov3* only in *M. bovis*. Interestingly, *M. bovis* shares IS*Mbov2* (homologous to IS*Mmy1*) and IS*Mbov3* (homologous to IS*1634*) with *M. mycoides* subsp. *mycoides* SC. Since these two mycoplasmas both infect bovines, it is possible that a horizontal transfer of the two IS elements might have occurred during an ancient co-infection of a cow. The lower number of polymorphisms found between IS*Mmy1* and IS*Mbov2*, and between IS*1634* and IS*Mbov3* (97% identity between *M. mycoides* subsp. *mycoides* SC and *M. bovis*) if compared to those found between IS*Mag1* and IS*Mbov1* (92% between *M. agalactiae* and *M. bovis*) may imply that the horizontal transfer events between the two bovine mycoplasmas are more recent than the divergent evolution of *M. agalactiae* and *M. bovis*.

In conclusion, *M. bovis*, like *M. mycoides* subsp. *mycoides* SC, is striking in its exceptionally high number of insertion elements (6–12 copies of IS*Mbov1*, 11–15 copies of IS*Mbov2*, 4–10 copies of IS*Mbov3* and 4–8 copies of IS*30*-like), representing approximately 60 kb or 6% of total genomic DNA, whose length was established to be 961 ± 18.9 kb for *M. bovis* [30]. This insertion sequence variability can be associated with rapid gene rearrangements that may confer improved genetic fitness of *M. bovis* towards specific host tissues.

Acknowledgements

The authors are grateful to Isabelle Dizier for technical help, to Dr. Sachse (BGVV, Jena, Germany), Dr. Ball (DARDNI, Belfast, UK), Dr. Blanchard (INRA, Bordeaux, France), Dr. Kempf (AFSSA, Ploufragan, France) and Dr. Boucher (Les Herbiers, France) for providing *M. bovis* isolates. This study was possible thanks to the grant from the Belgian 'Ministère de l'Agriculture' (convention 6039) and to the European COST Action 826.

References

[1] Adegboye, D.S., Halbur, P.G., Cavanaugh, D.L., Werdin, R.E., Chase, C.C.L., Miskimins, D.W. and Rosenbusch, R.F. (1995) Immunohistochemical and pathological study of *Mycoplasma bovis*-associated lung abscesses in calves. J. Vet. Diagn. Invest. 7, 333–337.

[2] Pinnow, C.C., Butler, J.A., Sachse, K., Hotzel, H., Timms, L.L. and Rosenbusch, R.F. (2001) Detection of *Mycoplasma bovis* in preservative-treated field milk samples. J. Dairy Sci. 84, 1640–1645.

[3] Burnens, A.P., Bonnemain, P., Bruderer, U., Schalch, L., Audige, L., Le Grand, D., Poumarat, F. and Nicolet, J. (1999) The seroprevalence of *Mycoplasma bovis* in lactating cows in Switzerland, particularly in the Republic and Canton of Jura. Schweiz. Arch. Tierh. 141, 455–460.

[4] Brice, N., Finlay, D., Bryson, D.G., Henderson, J., Mcconnell, W. and Ball, H.J. (2000) Isolation of *Mycoplasma bovis* from cattle in Northern Ireland, 1993 to 1998. Vet. Rec. 146, 643–644.

[5] Byrne, W.J., McCormack, R., Brice, N., Egan, J., Markey, B. and Ball, H.J. (2001) Isolation of *Mycoplasma bovis* from bovine clinical samples in the Republic of Ireland. Vet. Rec. 148, 331–333.

[6] Kusiluka, L.J., Ojeniyi, B. and Friis, N.F. (2000) Increasing prevalence of *Mycoplasma bovis* in Danish cattle. Acta Vet. Scand. 41, 139–146.

[7] Thomas, A., Ball, H., Dizier, I., Trolin, A., Bell, C., Mainil, J. and Linden, A. (2002) Isolation of mycoplasma species from the lower respiratory tract of healthy cattle and cattle with respiratory disease in Belgium. Vet. Rec. 151, 472–476.

[8] Bhugra, B. and Dybvig, K. (1993) Identification and characterization of IS*1138*, a transposable element from *Mycoplasma pulmonis* that belongs to the IS*3* family. Mol. Microbiol. 7, 577–584.

[9] Frey, J., Cheng, X., Kuhnert, P. and Nicolet, J. (1995) Identification and characterization of IS*1296* in *Mycoplasma mycoides* subsp. *mycoides* SC and presence in related mycoplasmas. Gene 160, 95–100.

[10] Calcutt, M.J., Lavrrar, J.L. and Wise, K.S. (1999) IS*1630* of *Mycoplasma fermentans*, a novel IS*30*-type insertion element that targets and duplicates inverted repeats of variable length and sequence during insertion. J. Bacteriol. 181, 7597–7607.
[11] Vilei, E.M., Nicolet, J. and Frey, J. (1999) IS*1634*, a novel insertion element creating long, variable-length direct repeats which is specific for *Mycoplasma mycoides* subsp. *mycoides* small-colony type. J. Bacteriol. 181, 1319–1323.
[12] Westberg, J., Persson, A., Pettersson, B., Uhlén, M. and Johansson, K.-E. (2002) ISMmy*1*, a novel insertion sequence of *Mycoplasma mycoides* subsp. *mycoides* small colony type. FEMS Microbiol. Lett. 208, 207–213.
[13] Pilo, P., Fleury, B., Marenda, M., Frey, J. and Vilei, E.M. (2003) Prevalence and distribution of the insertion element IS*Mag1* in *Mycoplasma agalactiae*. Vet. Microbiol. 92, 37–48.
[14] Lysnyansky, I., Ron, Y., Sachse, K. and Yogev, D. (2001) Intrachromosomal recombination within the *vsp* locus of *Mycoplasma bovis* generates a chimeric variable surface lipoprotein antigen. Infect. Immun. 69, 3703–3712.
[15] Ron, Y., Flitman-Tene, R., Dybvig, K. and Yogev, D. (2002) Identification and characterization of a site-specific tyrosine recombinase within the variable loci of *Mycoplasma bovis*, *Mycoplasma pulmonis* and *Mycoplasma agalactiae*. Gene 292, 205–211.
[16] Lysnyansky, I., Ron, Y. and Yogev, D. (2001) Juxtaposition of an active promoter to *vsp* genes via site-specific DNA inversions generates antigenic variation in *Mycoplasma bovis*. J. Bacteriol. 183, 5698–5708.
[17] Ball, H.J., Finlay, D. and Reilly, G.A. (1994) Sandwich ELISA detection of *Mycoplasma bovis* in pneumonic calf lungs and nasal swabs. Vet. Rec. 135, 531–532.
[18] Su, C.J., Dallo, S.F. and Baseman, J.B. (1990) Molecular distinctions among clinical isolates of *Mycoplasma pneumoniae*. J. Clin. Microbiol. 28, 1538–1540.
[19] Subramaniam, S., Bergonier, D., Poumarat, F., Capaul, S., Schlatter, Y., Nicolet, J. and Frey, J. (1998) Species identification of *Mycoplasma bovis* and *Mycoplasma agalactiae* based on the *uvrC* genes by PCR. Mol. Cell. Probes 12, 161–169.
[20] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1999) Current Protocols in Molecular Biology. Wiley, New York.
[21] Falquet, L., Pagni, M., Bucher, P., Hulo, N., Sigrist, C.J.A., Hofmann, K. and Bairoch, A. (2002) The PROSITE database, its status in 2002. Nucleic Acids Res. 30, 235–238.
[22] De Meirsman, C., Van Soom, C., Verreth, C., Van Gool, A. and Vanderleyden, J. (1990) Nucleotide sequence analysis of IS*427* and its target sites in *Agrobacterium tumefaciens* T37. Plasmid 24, 227–234.
[23] Mahillon, J. and Chandler, M. (1998) Insertion sequences. Microbiol. Mol. Biol. Rev. 62, 725–774.
[24] Hu, W.S., Hayes, M.M., Wang, R.Y., Shih, J.W. and Lo, S.C. (1998) High-frequency DNA rearrangements in the chromosomes of clinically isolated *Mycoplasma fermentans*. Curr. Microbiol. 37, 1–5.
[25] Cheng, X., Nicolet, J., Poumarat, F., Regalla, J., Thiaucourt, F. and Frey, J. (1995) Insertion element IS*1296* in *Mycoplasma mycoides* subsp. *mycoides* small colony identifies a European clonal line distinct from African and Australian strains. Microbiology 141, 3221–3228.
[26] Pitcher, D. and Hilbocus, J. (1998) Variability in the distribution and composition of insertion sequence-like elements in strains of *Mycoplasma fermentans*. FEMS Microbiol. Lett. 160, 101–109.
[27] Pettersson, B., Uhlen, M. and Johansson, K.E. (1996) Phylogeny of some mycoplasmas from ruminants based on 16S rRNA sequences and definition of a new cluster within the hominis group. Int. J. Syst. Bacteriol. 46, 1093–1098.
[28] Königsson, M.H., Bölske, G. and Johansson, K.E. (2002) Intraspecific variation in the 16S rRNA gene sequences of *Mycoplasma agalactiae* and *Mycoplasma bovis* strains. Vet. Microbiol. 85, 209–220.
[29] Thomas, A., Dizier, I., Linden, A., Mainil, J., Frey, J. and Vilei, E.M. (2004) Conservation of the *uvrC* gene sequence in *Mycoplasma bovis* and its use in routine PCR diagnosis. Vet. J. 168, 100–102.
[30] Tola, S., Idini, G., Rocchigiani, A.M., Manunta, D., Angioi, P.P., Rocca, S., Cocco, M. and Leori, G. (1999) Comparison of restriction pattern polymorphism of *Mycoplasma agalactiae* and *Mycoplasma bovis* by pulsed field gel electrophoresis. Zentralbl. Veterinarmed. [B] 46, 199–206.
[31] Thomas, A., Sachse, K., Dizier, I., Grajetzki, C., Farnir, F., Mainil, J.G. and Linden, A. (2003) Adherence to various host cell lines of *Mycoplasma bovis* strains differing in pathogenic and cultural features. Vet. Microbiol. 91, 101–113.