INFECTION AND IMMUNITY, Apr. 2002, p. 2220–2225 Vol. 70, No. 4
0019-9567/02/$04.00+0 DOI: 10.1128/IAI.70.4.2220–2225.2002

Extended Repertoire of Genes Encoding Variable Surface Lipoproteins in *Mycoplasma bovis* Strains

Sarit Nussbaum,[1] Inessa Lysnyansky,[1] Konrad Sachse,[2] Sharon Levisohn,[3] and David Yogev[1]*

Department of Membrane and Ultrastructure Research, The Hebrew University—Hadassah Medical School, Jerusalem 91120,[1] and Mycoplasma Unit, Kimron Veterinary Institute, Bet Dagan 50250,[3] Israel, and Federal Institute for Health Protection of Consumers and Veterinary Medicine, Division 4, Jena, Germany[2]

Received 10 August 2001/Returned for modification 4 December 2001/Accepted 8 January 2002

A genomic cluster of *vsp* genes was previously shown to mediate high-frequency phenotypic switching of surface lipoprotein antigens in the bovine pathogen *Mycoplasma bovis*. This study revealed that field strains of *M. bovis* possess modified versions of the *vsp* gene complex in which extensive sequence variations occur primarily in the reiterated coding sequences of the *vsp* structural genes. These findings demonstrate that there is a vastly expanded potential for antigenic variation within populations of this organism.

An important microbial strategy for host adaptation and evasion of immune responses is the maintenance of diversity in propagating populations. This is commonly manifest in structurally variant forms of major coat proteins that are transiently expressed as a result of random or programmed molecular events (4, 6, 22, 23, 27, 28). In many cases, large families of related genes organized in genomic loci are utilized. Homologous recombination, gene conversions, gene duplications, additions or deletions of tandem repetitive units, and DNA inversions are mechanisms that are frequently used to regulate the phase-variable expression of these genes at a high frequency (4, 6, 11, 12, 13, 14, 15, 19, 22, 23, 30).

Mycoplasma bovis, a bovine pathogen (8, 16, 25), contains an elaborate genetic system in which multiple related but divergent genes encoding variable surface lipoproteins (Vsps) undergo spontaneous on/off switching (9, 10). The Vsp antigens have been shown to be highly immunogenic and to contain adhesive structures in the repetitive domains of their molecules (1, 21). High-frequency site-specific DNA inversions that occur within the *vsp* locus dictate the degree of Vsp diversification within propagating populations by determining which *vsp* gene is expressed in a given cell (9, 12). The extent of Vsp antigenic variation also depends strongly on the number of *vsp* genes available, particularly with respect to the generation of combinatorial diversity. Recently, we have shown that the *vsp* gene repertoire undergoes changes by intrachromosomal recombination within the *vsp* locus between closely related *vsp* genes, which leads to the generation of a chimeric *vsp* gene (11). The extent of the natural *vsp* gene reservoir in *M. bovis* is not known, but its size and modulation may significantly affect the adaptive ability of this species. The present study was undertaken to examine the extents of the *vsp* gene repertoire in different field strains of *M. bovis*.

Eight strains of *M. bovis* isolated from distinct sites (lung, joint, trachea, nose, and milk) in bovine hosts with various clinical presentations (mastitis, pneumonia, arthritis, and healthy) (20) were examined. Genomic DNAs of these strains were extracted, digested with the *Hin*dIII restriction enzyme, and subjected to Southern blot hybridization under low-stringency conditions. A 1.5-kb *Hin*dIII fragment carrying the *vspA* structural gene and its highly conserved *vsp* upstream region (10) and two oligonucleotides representing unique repetitive coding sequences present only in the *vspE* gene (R_E1) or only in the *vspF* gene (R_F2) of type strain PG45 (10) were used as probes. Complex hybridization profiles for multiple *Hin*dIII genomic fragments with different intensities and of different sizes were obtained for the strains examined with the *vspA* gene probe (Fig. 1A). The variations in the *vsp*-related fragments among strains (2, 17) presumably reflect the presence of distinct configurations of the *vsp* genomic locus due to high-frequency site-specific DNA inversions, which have been shown to occur in that locus (11, 12).

Interestingly, however, the R_E1 region, which is repeated in tandem eight times within the *vspE* structural gene of the PG45 strain and is localized on a single 1.2-kb *Hin*dIII genomic fragment (Fig. 1B, lane 1) (10), was not observed in five different strains (Fig. 1B, lanes 2 to 5 and 8), and in one strain it was identified in several *Hin*dIII genomic fragments (Fig. 1B, lane 7). The R_F2 region, which is repeated seven times within the *vspF* structural gene and is localized on a single 1.8-kb *Hin*dIII fragment in the PG45 strain (Fig. 1C, lane 1) (10), was missing in one strain (Fig. 1C, lane 8). Collectively, the data suggest that extensive variations in the *vsp* reiterated coding sequences might occur among strains.

One strain, strain 422, which was isolated from the lung of a calf with pneumonia and in which both the R_E1 and R_F2 repetitive domains were missing (Fig. 1B and C, lanes 8), was chosen for further analysis. The *vsp* genomic locus of this strain was cloned and sequenced. A cluster of 11 Vsp-related open reading frames (ORFs) that were not all similarly oriented was identified (Fig. 2). The nucleotide sequences of the 11 *vsp* genes and the deduced proteins were compared to the nucleotide sequences of the 13 *vsp* counterparts and the deduced proteins in the PG45 strain (10). The *vsp* genes of the two strains were found to have two highly homologous domains.

* Corresponding author. Mailing address: Department of Membrane and Ultrastructure Research, The Hebrew University—Hadassah Medical School, Jerusalem 91120, Israel. Phone: 972-2-6758176. Fax: 972-2-6784010. E-mail: yogev@cc.huji.ac.il.

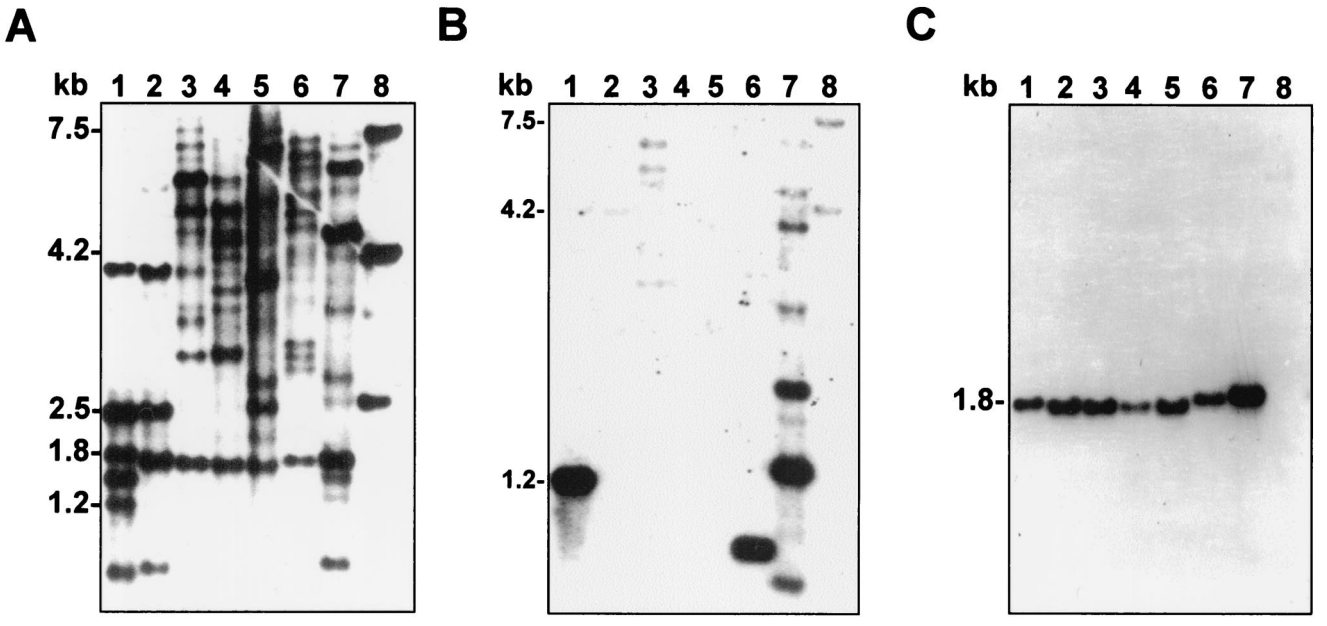

FIG. 1. Identification of multiple *vsp*-related genomic fragments in *M. bovis* strains. Four micrograms of chromosomal DNA of each strain was digested with the *Hin*dIII restriction enzyme, subjected to Southern blot hybridization, and probed with the ^{32}P-labeled *vspA* gene (A), with oligonucleotide R_E1 (B), and with oligonucleotide R_F2 (C). The conditions used for oligonucleotide labeling, DNA labeling, and Southern blot hybridization have been described elsewhere (9, 10). The *M. bovis* strains used included type strain PG45 (lane 1), D490 (lane 2), D857 (lane 3), D860 (lane 4), D1098 (lane 5), D1499 (lane 6), D1517 (lane 7), and 422 (lane 8). The positions of molecular size markers are indicated on the left.

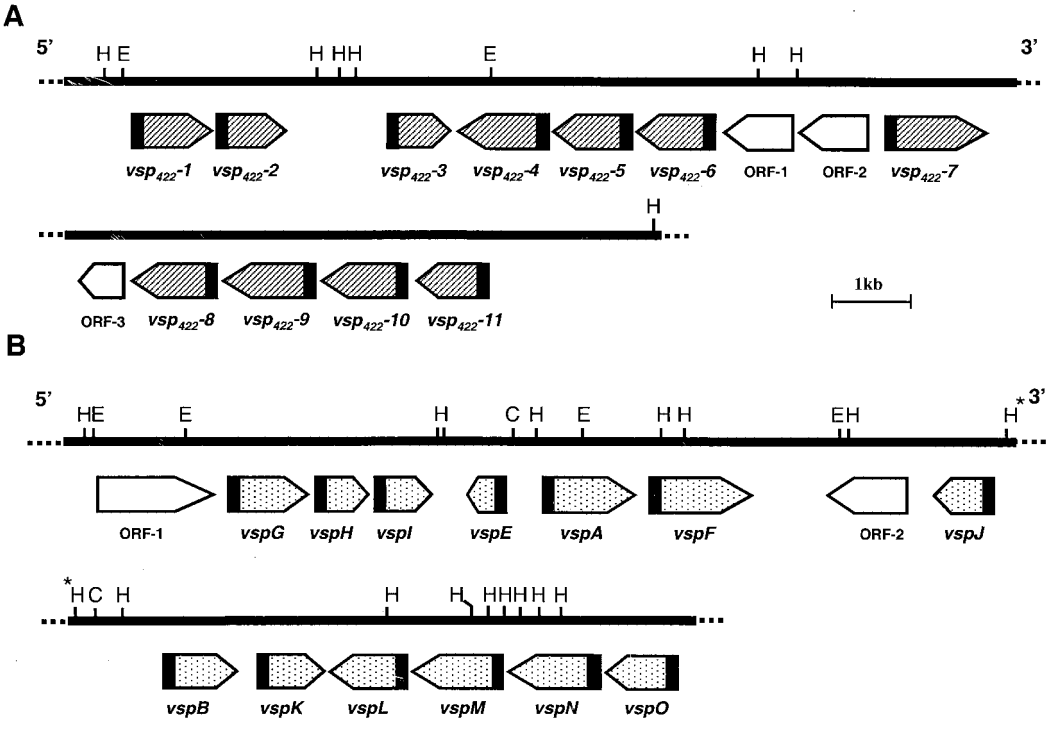

FIG. 2. Schematic representation, partial restriction map, genomic organization, and comparison of the *vsp* locus of *M. bovis* strain 422 and the *vsp* locus of *M. bovis* type strain PG45. (A) *vsp* locus of strain 422. The solid line represents a 16.7-kb *M. bovis* DNA insert obtained from a recombinant bacteriophage designated λMb422-1 isolated from a genomic library constructed with the phage vector λGEM-12 (*Xho*I half arms; Promega). The library was screened with the *vspA* gene as previously described (9, 10). The positions of *Hin*dIII (H) and *Eco*RI (E) restriction sites are indicated. The locations and directions of 11 Vsp ORFs are indicated by labeled arrows. The positions of three non-Vsp-related ORFs (ORF-1 to ORF-3) are also indicated by labeled arrows. The highly homologous *vsp* upstream regions, as well as the conserved N-terminus-encoding regions, are indicated by the cross-hatched portions of the arrows. The nucleotide sequences of *vsp* genes 1 to 11 of *M. bovis* strain 422 have been assigned GenBank accession numbers AF406972 to AF406982, respectively. (B) *vsp* locus of *M. bovis* type strain PG45, as previously described (10). The position of the *Cla*I (C) restriction site is also indicated.

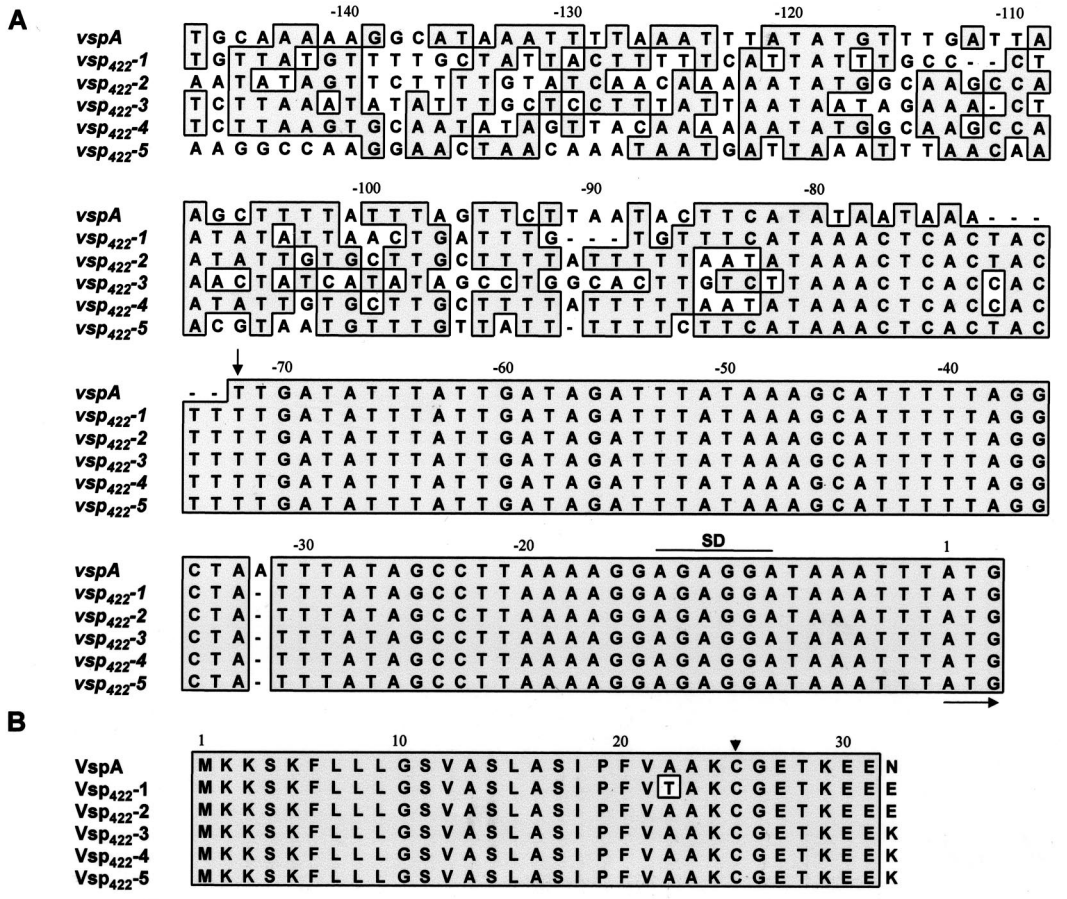

FIG. 3. Sequence alignments for *vsp* 5′ upstream regions (A) and Vsp-N-terminal regions (B). Five representatives of the *vsp* gene family of *M. bovis* strain 422 (*vsp*$_{422}$*-1* to *-5*) and the *vspA* gene, a representative *vsp* gene of *M. bovis* type strain PG45, were compared. The alignment was constructed with the MacVector 6.0 software program. The numbers above the sequences indicate positions relative to the initiation codon. Nucleotides representing a putative ribosome-binding site (SD) are overlined. The initiation codon (ATG) is indicated by a horizontal arrow. The division of the *vsp* 5′ upstream region into two distinct cassettes is indicated by a vertical arrow at nucleotide position −72 (10). Identical nucleotides or amino acid resides are indicated by shaded boxes. The single Cys residue in the Vsp lipoprotein box is indicated by an arrowhead.

One of these domains is a 5′ upstream region that can be divided into two cassettes. A 72-bp region upstream of the ATG initiation codon contains a putative ribosome binding site and exhibits 99% homology in all *vsp* genes, while the second cassette, which is upstream of the first cassette, is more divergent (Fig. 3A). The second domain is the N-terminal region encoding 32 amino acids that exhibit 98% identity in the Vsps and contain a typical prokaryotic lipoprotein signal peptide. The protein sequence begins with three positively charged Lys residues, followed by a core of 20 hydrophobic amino acids, and it ends with the tetrapeptide Ala-Ala-Lys-Cys (Fig. 3B).

Interestingly, in contrast to the highly conserved nature of the *vsp* 5′ upstream region and of the N-terminal domain, there is considerable divergence in the deduced amino acid sequences of the Vsp proteins between the two strains. In both *M. bovis* PG45 and 422, most of the Vsp molecules contain an unusual motif consisting of reiterated coding sequences extending from the N terminus to the C terminus of the protein chain that create a periodic polypeptide structure (Fig. 4) (10). The majority of these repeated sequences are arranged in tandem domains that comprise up to 80% of the Vsp molecule. However, the substantial sequence divergence among individual Vsps within each family and the sequence divergence between the two families are the result of at least 30 repetitive units consisting of different amino acid sequences of different lengths (range, 4 to 127 amino acids), which are distributed within the Vsp proteins of the two families (Table 1) (10). Within the *vsp* gene family of strain 422, there are repeats that are present in only one Vsp (e.g., R_1-2, R_3-1, R_4-2, R_5-2, R_5-3, R_7-1, R_9-3, R_9-4, R_{10}-2, and R_{10}-3), whereas other repeats occur at variable locations in several Vsps (e.g., R_1-1, R_3-2, R_4-1, R_6-1, R_6-2, and R_{10}-2) (Fig. 4 and Table 1).

Importantly, a comparison of all *vsp* reiterated coding sequences in the two *M. bovis* strains revealed the presence of strain-specific repetitive sequences. Nine repeats were found only in strain 422 (R_1-2, R_4-2, R_5-2, R_5-3, R_7-1, R_9-3, R_9-4, R_{10}-2, and R_{10}-3, consisting of 6, 77, 5, 10, 127, 16, 16, 10, and 15 amino acids, respectively), and 13 repeats were found only in the PG45 strain (R_A-2, R_A-3, R_A-4, R_E-1, R_F-1, R_F-2, R_G-2, R_G-4, R_J-1, R_K-2, R_K-3 R_N-2, and R_O-2, consisting of 6, 8, 8, 6, 84, 10, 10, 11, 26, 6, 11, 87, and 12 amino acids, respectively) (Table 1) (10). Other repeats exhibited partial homology in the

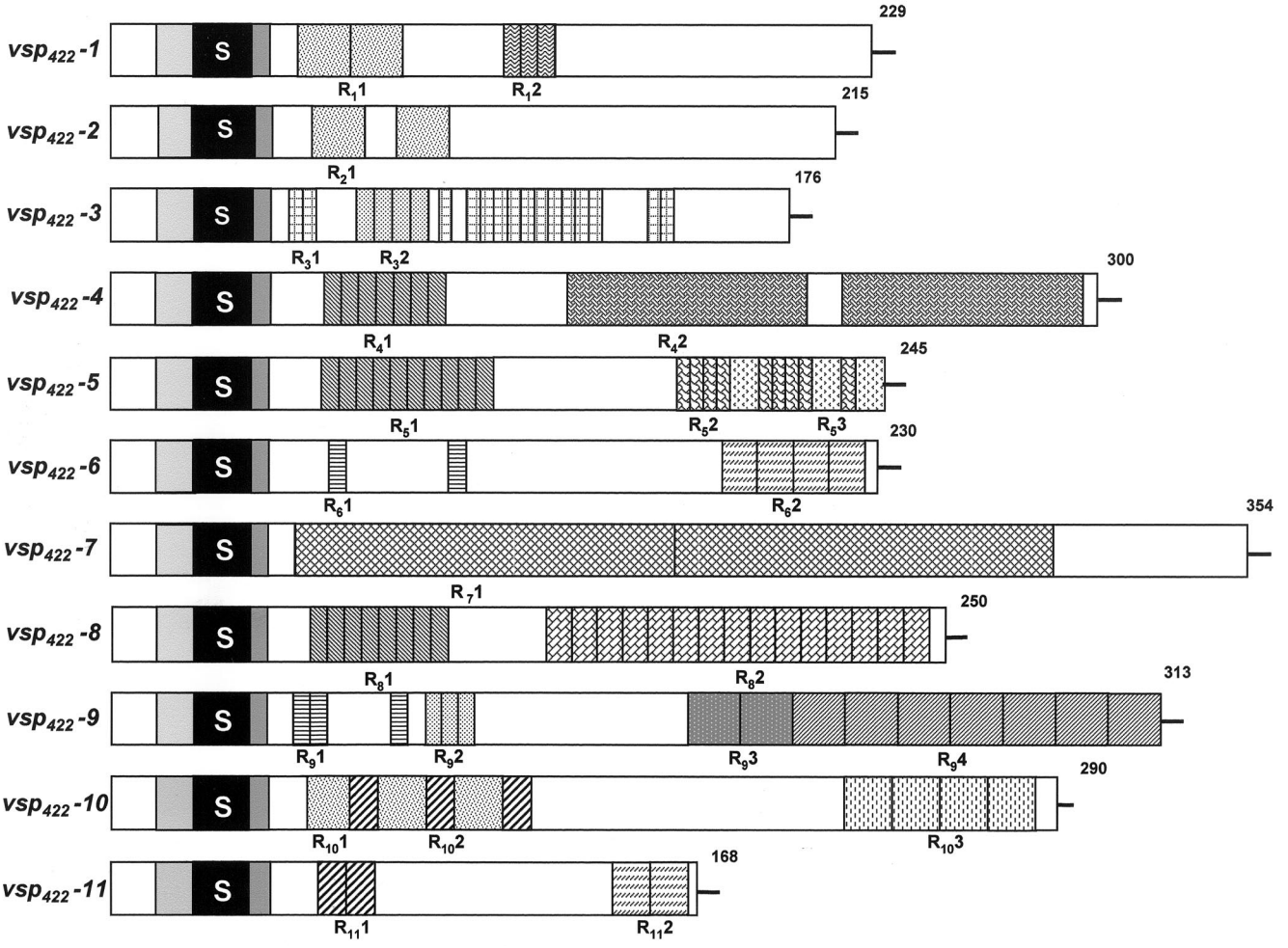

FIG. 4. Structural features and comparison of *vsp* genes and Vsp products of *M. bovis* strain 422. The structures of the *vsp* genes and the predicted Vsp proteins are represented schematically by aligned boxes. A highly conserved region upstream of each *vsp* gene is represented by the first gray box. The second box (solid box labeled S) represents a highly homologous 75-bp DNA sequence encoding a conserved prolipoprotein signal peptide. A sequence of six amino acids common to all Vsps is represented by the third box (dark gray). In-frame reiterated coding sequences extending from the N termini to the C termini of the Vsp proteins and encoding periodic amino acid sequences are represented by different kinds of boxes. Distinctive repetitive domains within each Vsp are labeled with R and the number of the corresponding *vsp* gene. Repetitive units present in more than one Vsp molecule are represented similarly. The number to the right of each Vsp indicates the length of the Vsp polypeptide chain.

two strains (for example, R_3-1), while five repeats were found in both strains (R_3-2, R_4-1, R_6-1, R_6-2, and R_8-2, consisting of 6, 6, 6, 11, and 8 amino acids, respectively). Notably, however, the distribution of the repeats found in both strains within individual Vsps, as well as their copy numbers, varied considerably between the two *vsp* gene families (Fig. 4) (10). Together, the presence of strain-specific *vsp* repetitive coding sequences and the distribution of shared repeats among *vsp* genes may generate an amplified array of Vsp phenotypes that differ in their antigenic characteristics.

The results of this study revealed expanded possibilities for adaptive surface variation for the *vsp* gene system. Not only do the phase and size variations among Vsps create extensive potential for antigenic and structural diversification on the surface of *M. bovis*, but also the extent of the *vsp* gene repertoire that can be expressed in a population provides an additional factor that may profoundly affect the possible variation. Documented examples of increased repertoires of genes encoding variable antigens include the *vlp* system of the swine pathogen *Mycoplasma hyorhinis* and the *vsa* system of the murine pathogen *Mycoplasma pulmonis*. In *M. hyorhinis* the high-passage avirulent GDL strain possesses six *vlp* genes (*vlpA* to *vlpF*), while clonal isolates of the pathogenic strain SK76 contain three or seven *vlp* genes (*vlpA* to *vlpC* or *vlpA* to *vlpG*) (5, 29). *M. pulmonis* strain UAB CTIP contains 7 *vsa* genes, while *M. pulmonis* strain KD735-15 contains 11 *vsa* genes (3, 24). In contrast, with the exception of the conserved Vsp N-terminal region, none of the *vsp* genes of *M. bovis* strain PG45 was identical to a *vsp* gene of *M. bovis* strain 422. Thus, extended antigenic phenotypes within a propagating population appear to be a selective and heritable feature of these organisms and probably contribute significantly to the successful persistence of chronic infections caused by pathogenic mycoplasmas in their natural hosts (18, 25, 28).

TABLE 1. Repetitive sequences in *M. bovis* strain 422 Vsp proteins

Protein[a]	Length of ORF (no. of amino acids)	Repeat	Repeat length (no. of amino acids)	No. of Vsp repeats	Repeat amino acid sequence[b]	Other Vsp(s) in which repeat is present	Vsp(s) of strain PG45 in which repeat or part of repeat is present[c]
Vsp_{422}-1	229	R_1-1	16	2	NTDPGKNPGGDKNPGG(Q)	Vsp_{422}-2, Vsp_{422}-10	VspG (R_G-1), VspK (R_K-1) [PGGDKN], VspN (R_N-1) [NTDPGKNPGG][d]
		R_1-2	6	3	PEGNKT		
Vsp_{422}-2	215	R_2-1	16	2	NTDPGKNPGGDKNPGG(S)	Vsp_{422}-1, Vsp_{422}-10	VspG (R_G-1), VspK (R_K-1) [PGGDKN], VspN (R_N-1) [NTDPGKNPGG][d]
Vsp_{422}-3	176	R_3-1	4	15	DKPA(G)(S)		VspJ (R_J-1) [KEEKKPEADKPADKQPGDDM(I)KKDNDK][d]
		R_3-2	6	4	PEGEKK	Vsp_{422}-9	VspH (R_H-2) [PEGEKK][d]
Vsp_{422}-4	300	R_4-1	6	7	PGENKT	Vsp_{422}-5, Vsp_{422}-8	VspA (R_A-1), VspG (R_G-3), VspH (R_H-1), VspO (R_O-1) [PGENKT][d,e]
		R_4-2	77	2	KKTDIGKIDENKKKELLK ELDKQFSINPDYNVIEK ALQKYFKEIKKIDFLVQ GSDKKLEIAAKGDSPK FKGTIELKK		
Vsp_{422}-5	245	R_5-1	6	10	PGENKT	Vsp_{422}-4, Vsp_{422}-8	VspA (R_A-1), VspG (R_G-3), VspH (R_H-1), VspO (R_O-1) [PGENKT][d,e]
		R_5-2	5	9	AGQSS		
		R_5-3	10	3	AGQNKAGQG(S)S		
Vsp_{422}-6	230	R_6-1	6	2	PGGDKN	Part of Vsp_{422}-1, Vsp_{422}-2, Vsp_{422}10	
						Vsp_{422}-9	VspG (R_G1), VspK (R_K−1) [PGGDKN][d]
		R_6-2	11	4	QGTEANSGKKV	Vsp_{422}-11	VspM (R_M-2) [QGTEANSGKKV][d]
Vsp_{422}-7	354	R_7-1	127(123)[f]	2	KEEKKPEADKP[TNKP][f]AD KQPGDDMKKDNDKSK SDKDKNEKDMTDKDK KDQEKSEKDKVENTKI DDSKINKNLGSFSKNSI TNAVRQKDIRDKIVGI LKTQNFNLDVNYEKH EARVTLSNGQVIIFTF	Vsp_{422}-3 (DKPA)	VspJ (R_J-1) [KEEKKPEADKPADKQP GDDMKKDNDK][d]
Vsp_{422}-8	250	R_8-1	6	8	PGENKT	Vsp_{422}-4, Vsp_{422}-5	VspA (R_A-1), VspG (R_G-3), VspH (R_H-1), VspO (R_O-1) [PGENKT][d,e]
		R_8-2	8	15	PDQGTPT(A)K		VspI (R_I-1) [PDQGTPAN][d]
Vsp_{422}-9	313	R_9-1	6	3	PGGDKN	Part of Vsp_{422}-1, Vsp_{422}-2, Vsp_{422}-10	
						Vsp_{422}-6	VspG (R_G-1), VspK (R_K-1) [PGGDKN][d]
		R_9-2	6	3	PEGE(D)KK	Vsp_{422}-3	VspH (R_H-2) [PEGEKK][d]
		R_9-3	16	2	KPRQGTDAKAGQSPAA(G)		
		R_9-4	16	7	KSGQSTDAKSGQSPAG		
Vsp_{422}-10	290	R_{10}-1	16	3	NTE(D)PGKNPGGDKNPGG	Vsp_{422}-1, Vsp_{422}-2	VspG (R_G-1), VspK (R_K-1) [PGGDKN], VspN (R_N-1) [NTE(D)PGKNPGG][d]
		R_{10}-2	10	3	NTEPGKNPSE	Vsp_{422}-11	
		R_{10}-3	15	4	QPGKPGATTQPSGAV		
Vsp_{422}-11	168	R_{11}-1	10	2	NTE(D)PGKNOSE(Q)	Vsp_{422}-10	
		R_{11}-2	11	2	QGTEANSGKKV	Vsp_{422}-6	VspM (R_M-2) [QGTEANSGKKV][d]

[a] Data from this study.
[b] Parentheses indicate amino acid substitutions within the repeats.
[c] The sequences in brackets are the parts of the repeats in Vsps of strain PG45.
[d] Data from reference 10.
[e] Data from reference 9.
[f] For this protein, the amino acids in brackets are missing in the second repeat.

The repetitive surface-exposed C-terminal ends of the Vsp proteins were shown to possess immunogenic epitopes and adhesive structures, suggesting that these molecules may play a role as complex adherence-mediating regions in pathogenesis (21). The finding that variations among individual Vsps of both strains were localized solely within Vsp repetitive regions may suggest that strains of the same species were subjected to different selective pressures by their hosts during evolution. Notably, two ORFs embedded in the *vsp* locus of strain PG45 exhibit high homology to mobile genetic elements. For example, ORF-1 exhibits homology to insertion sequence IS*4* and ORF-2 exhibits homology to insertion sequence IS*30* of *Escherichia coli* (10). The existence of *M. bovis* strains carrying modified versions of the *vsp* gene system and the presence of ORFs associated with mobile elements or mobility functions raise an intriguing question about the possibility of natural gene transfer among cells that are in direct contact in propagating populations or even during infection within the natural host (26). The presence of multiple regions with a high level of sequence similarity upstream of the *vsp* genes and within the

vsp coding regions would allow modulation of the *vsp* gene repertoire by recombination processes (6, 11, 12, 15).

Analyses of genetic systems that mediate antigenic variation in several pathogenic mycoplasmas (3, 7, 12, 14, 24, 30) have revealed diverse mechanistic features of expression and structural variations. The presence of an extended repertoire of genes encoding variable surface antigens in strains of the same species provides another important means of surface diversification and underscores the efficient way that these wall-less minute microorganisms manipulate their limited genetic material for maximum adaptive flexibility in the host.

This study was supported in part by the German-Israeli Foundation for Scientific Research and Development, by research grant IS-3126-99 from the United States-Israel Binational Agricultural Research and Development Fund, and by the Israel Academy of Sciences and Humanities Foundation.

REFERENCES

1. **Behrens, A., M. Heller, H. Kirchhoff, D. Yogev, and R. Rosengarten.** 1994. A family of phase- and size-variant membrane surface lipoprotein antigens (Vsps) of *Mycoplasma bovis*. Infect. Immun. **62:**5075–5084.
2. **Beier, T., H. Hotzel, I. Lysnyansky, C. Grajetzki, M. Heller, B. Rabeling, D. Yogev, and K. Sachse.** 1998. Intraspecies polymorphism of *vsp* genes and expression profiles of variable surface protein antigens (Vsps) in field isolates of *Mycoplasma bovis*. Vet. Microbiol. **63:**189–203.
3. **Bhugra, B., L. L. Voelker, N. Zou, H. Yu, and K. Dybvig.** 1995. Mechanism of antigenic variation in *Mycoplasma pulmonis*: interwoven, site-specific DNA inversions. Mol. Microbiol. **18:**703–714.
4. **Borst, P., and D. R. Greaves.** 1987. Programmed gene rearrangements altering gene expression. Science **235:**658–667.
5. **Citti, C., R. Watson-McKown, M. Droesse, and K. S. Wise.** 2000. Gene families encoding phase- and size-variable surface lipoproteins of *Mycoplasma hyorhinis*. J. Bacteriol. **182:**1356–1363.
6. **Dybvig, K.** 1993. DNA rearrangements and phenotypic switching in prokaryotes. Mol. Microbiol. **10:**465–471.
7. **Glew, M. D., N. Baseggio, P. F. Markham, G. F. Browning, and I. D. Walker.** 1998. Expression of the pMGA genes of *Mycoplasma gallisepticum* is controlled by variation in the GAA trinucleotide repeat lengths within the 5′ noncoding regions. Infect. Immun. **66:**5833–5841.
8. **Jasper, D. E.** 1987. Bovine mastitis due to mycoplasma. Rev. Sci. Tech. Off. Int. Epizoot. **6:**801–807.
9. **Lysnyansky, I., R. Rosengarten, and D. Yogev.** 1996. Phenotypic switching of variable surface lipoproteins in *Mycoplasma bovis* involves high-frequency chromosomal rearrangements. J. Bacteriol. **178:**5395–5401.
10. **Lysnyansky, I., K. Sachse, R. Rosenbusch, S. Levisohn, and D. Yogev.** 1999. The *vsp* locus of *Mycoplasma bovis*: gene organization and structural features. J. Bacteriol. **181:**5734–5741.
11. **Lysnyansky, I., Y. Ron., K. Sachse, and D. Yogev.** 2001. Intrachromosomal recombination within the *vsp* locus of *Mycoplasma bovis* generates a chimeric variable surface lipoprotein antigen. Infect. Immun. **69:**3703–3712.
12. **Lysnyansky, I., Y. Ron, and D. Yogev.** 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.
13. **Meyer, T. F., C. P. Gibbs, and R. Haas.** 1990. Variation and control of protein expression in *Neisseria*. Annu. Rev. Microbiol. **44:**451–477.
14. **Noormohammadi, A. H., P. F. Markham, A. Kanci, K. G. Whithear, and G. F. Browning.** 2000. A novel mechanism for control of antigenic variation in the haemagglutinin gene family of *Mycoplasma synoviae*. Mol. Microbiol. **35:**911–923.
15. **Petes, T. D., and C. W. Hill.** 1988. Recombination between repeated genes in microorganisms. Annu. Rev. Genet. **22:**147–168.
16. **Pfützner, H., and K. Sachse.** 1996. *Mycoplasma bovis* as an agent of mastitis pneumonia, arthritis and genital disorders in cattle. Rev. Sci. Tech. Off. Int. Epizoot. **15:**1477–1494.
17. **Poumarat, F., D. Le Grand, M. Solsona, R. Rosengarten, and C. Citti.** 1999. Vsp antigens and *vsp*-related DNA sequences in field isolates of *Mycoplasma bovis*. FEMS Microbiol. Lett. **173:**103–110.
18. **Razin, S., D. Yogev, and Y. Naot.** 1998. Molecular biology and pathogenicity of mycoplasmas. Microbiol. Mol. Biol. Rev. **62:**1094–1156.
19. **Robertson, B. D., and T. F. Meyer.** 1992. Antigenic variation in bacterial pathogens, p. 61–73. *In* C. W. Hormaeche, C. W. Penn, and C. J. Smyth (ed.), Molecular biology of bacterial infection, vol. 49. Cambridge University Press, Cambridge, United Kingdom.
20. **Rosengarten, R., A. Behrens, A. Stetefeld, M. Heller, M. Ahrens, K. Sachse, D. Yogev, and H. Kirchhoff.** 1994. Antigen heterogeneity among isolates of *Mycoplasma bovis* is generated by high-frequency variation of diverse membrane surface proteins. Infect. Immun. **62:**5066–5074.
21. **Sachse, K., J. H. Helbig, I. Lysnyansky, C. Grajetzki, W. Muller, E. Jacobs, and D. Yogev.** 2000. Epitope mapping of immunogenic and adhesive structures in repetitive domains of *Mycoplasma bovis* variable surface lipoproteins. Infect. Immun. **68:**680–687.
22. **Saunders, J. R.** 1986. Genetic basis of phase and antigenic variation in bacteria, p. 57–76. *In* T. H. Birkbeck and C. W. Penn (ed.), Antigenic variation in infectious diseases. Society for General Microbiology, IRL Press, Oxford, United Kingdom.
23. **Seifert, H. S., and M. So.** 1988. Genetic mechanisms of bacterial antigenic variation. Microbiol. Rev. **52:**327–336.
24. **Shen, X., J. Gumulak, H. Yu, C. Todd French, N. Zou, and K. Dybvig.** 2000. Gene rearrangements in the *vsa* locus of *Mycoplasma pulmonis*. J. Bacteriol. **182:**2900–2908.
25. **Simecka, J. W., J. K. Davis, M. K. Davidson, S. E. Ross, C. T. K.-H. Stadtländer, and G. H. Cassell.** 1992. Mycoplasma diseases of animals, p. 391–416. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
26. **Veal, A. D., H. W. Stokes, and G. Daggard.** 1992. Genetic exchange in natural microbial communities, p. 383–430. *In* K. C. Marshall (ed.), Advances in microbial ecology, vol. 12. Plenum Press, New York, N.Y.
27. **Wise, K. S., D. Yogev, and R. Rosengarten.** 1992. Antigenic variation, p. 473–489. *In* J. Maniloff, R. N. McElhaney, L. R. Finch, and J. B. Baseman (ed.), Mycoplasmas: molecular biology and pathogenesis. American Society for Microbiology, Washington, D.C.
28. **Wise, K. S.** 1993. Adaptive surface variation in mycoplasmas. Trends Microbiol. **1:**59–63.
29. **Yogev, D., R. Watson-McKown, R. Rosengarten, J. Im, and K. S. Wise.** 1995. Increased structural and combinatorial diversity in an extended family of genes encoding Vlp surface proteins of *Mycoplasma hyorhinis*. J. Bacteriol. **177:**5636–5643.
30. **Yogev, D., R. Rosengarten, R. Watson-McKown, and K. S. Wise.** 1991. Molecular basis of *Mycoplasma* surface antigenic variation: a novel set of divergent genes undergo spontaneous mutation of periodic coding regions and 5′ regulatory sequences. EMBO J. **10:**4069–4079.

Editor: V. J. DiRita