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Genomic Maps of Some Strains within the *Mycoplasma mycoides* Cluster

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Genomic restriction maps for the small colony (SC) strains (PG1, KH_3J, Gladysdale, and V5) of *Mycoplasma mycoides* subsp. *mycoides* (the agent of contagious bovine pleuropneumonia) and for *Mycoplasma* strain PG50 (classified as bovine serogroup 7), with respective sizes of 1,280, 1,280, 1,260, 1,230, and 1,040 kbp, were compared with the map (1,200 kbp) for a large colony strain (Y goat) of *M. mycoides* subsp. *mycoides*. The number and order of all mapped restriction sites were fully conserved in the SC genomes, as were the approximate positions of mapped loci. A number of these restriction sites in the Y genome and some, but fewer, in the PG50 genome appeared to be conserved. The SC and large colony strains shared conservation in the relative positions of the mapped loci, except for *rpoC*.

The use of pulsed-field gel electrophoresis for the separation of large restriction fragments has made possible the construction of physical maps for several procaryotic genomes, including two large colony (LC) strains of *Mycoplasma mycoides* subsp. *mycoides*, the Y goat strain, and GC1176-2 (10, 19a, 20). These show congruence in the gene loci and many of the mapped restriction sites when three divergences totalling 180 kbp are allowed for in the larger genome of GC1176-2. *M. mycoides* subsp. *mycoides* also includes small colony (SC) strains, and the subspecies is part of a larger grouping of ruminant pathogens called the *M. mycoides* cluster (4). Here, we compare the map for *M. mycoides* subsp. *mycoides* Y goat with the genomic restriction maps and placement of various gene loci for four SC strains of *M. mycoides* subsp. *mycoides* and one strain, PG50, of bovine serogroup 7, another member of the cluster.

M. mycoides subsp. *mycoides* SC strains PG1, KH_3J, Gladysdale, and V5 were from the culture collection of G. Cottew, whereas PG50 was obtained from C. Christiansen. Preparation and analysis of DNA in agarose to construct restriction maps were as previously described (3, 8–10). Gene loci were detected by probing Southern blots with cloned DNAs (Table 1). Sizes of fragments up to 500 kbp were measured against bacteriophage λ DNA multimers as markers, whereas for larger fragments the markers were yeast chromosomal DNA molecules (Bio-Rad Laboratories) and *Bss*HII and *Apa*I digests of DNA from *M. mycoides* subsp. *mycoides* Y goat (10) and GC1176-2 (19a). The sizes of the single *Bss*HII fragments, representing the complete genomes, from the DNA of the SC strains were in reasonable agreement with the sums of the sizes of the fragments in the other digests. From these values, the sizes of the genomes fell in a range of about 1,230 to 1,280 kbp compared with values of 1,200 and 1,380 kbp for map sizes of LC *M. mycoides* Y goat and GC1176-2, respectively. The size of the PG50 genome was not measured as a single fragment, but the sums of the fragment sizes for each of the digests were in reasonable agreement with the rounded average value of 1,040 kbp shown in Fig. 1. Previous estimates from DNA renaturation kinetics gave genome size values of 500 or 570 MDa (810 or 923 kbp) for *M. mycoides* subsp. *mycoides* PG1 and 560 MDa (907 kbp) for PG50 (13). Thus, the data provided further examples of considerably larger values for mycoplasma genome sizes when measured by pulsed-field gel electrophoresis compared with DNA renaturation and add to evidence that the genomes of members of the family *Mycoplasmataceae* are diverse in size (8, 15).

Construction of maps followed the rationale used for the restriction map of the *M. mycoides* susbp. *mycoides* Y goat genome (10). The latter was shown in Fig. 1 with additional functional loci for comparison with maps of the other strains. It was observed, but not indicated on the maps in Fig. 1, that *Apa*I did not digest PG50 or SC *M. mycoides* subsp. *mycoides* DNA, whereas there are two *Apa*I sites in the genomes of LC *M. mycoides* subsp. *mycoides* Y goat and GC1176-2 (10, 19a). A procedure (3) for labeling restriction fragments in agarose blocks did not detect additional small fragments from single digests of the DNA from the SC strains or PG50. However, an additional *Kpn*I fragment of 4 kbp was detected from the DNAs of LC strains Y goat and GC1176-2. It mapped between *Kpn*I fragments B and C (10, 19a, 20) in both genomes.

The response of the restriction fragments from the DNAs of the various strains to probing with cloned DNA for several mycoplasma genes (Table 1) allowed for definition of the loci shown on the maps in Fig. 1. Several of the probes reacted with fragments from either side of a restriction site. In each case, the restriction endonuclease concerned also cleaved the insert DNA in the probe. Where tested, the fragments of the insert separated by such digestion each reacted with a different one of the two genomic DNA fragments. This was consistent with the restriction site within the cloned DNA probe being conserved within the genomic DNA sequence responding to it. The loci defined by such probes were designated in Fig. 1 by arrows pointing to the particular restriction sites concerned, as an indication that the locus included, or was immediately adjacent to, the site. Other loci were shown as lying within the smallest restriction fragment defined by reaction to the probe. The order of the loci within fragments was not determined.

In Fig. 1, the circular genomes were represented as linear maps with the zero coordinate for each map at the conserved *Bgl*I site within the $tRNA^{Leu}$ locus (*leuT*). The left-to-right

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TABLE 1. Cloned DNA used for probing digests of mycoplasma DNA

Cloned DNA probe (reference)[a]	Defined gene product	Locus	Source of cloned DNA
pMC5 (1)	rRNA	*rrn*	*M. capricolum*
pMM22 (17)	$tRNA^{Arg}$	*argT*	*M. mycoides* subsp. *capri*
pMM25 (17)	9 tRNAs	*trnA*	*M. mycoides* subsp. *capri*
pTZ19Rgly (16)	$tRNA^{Gly}$	*glyT*	*M. mycoides* subsp. *capri*
pTZ19Rleu (5)	$tRNA^{Leu}$	*leuT*	*M. mycoides* subsp. *capri*
Insert in M13mp9 (18)	4 tRNAs	*trnC*	*M. mycoides* subsp. *capri*
pMYC405 (11)	Most of ATPase	*atp*	*Mycoplasma* strain PG50
pMYC708 (12)	Part of RNA polymerase β subunit	*rpoC*	*Mycoplasma* strain PG50
pMYC714 (12)	Serine hydroxymethyltransferase	*glyA*	*Mycoplasma* strain PG50
pMCB1088 (6)	13 ribosomal proteins	*rpn*	*M. capricolum*

[a] Plasmid and viral DNAs were kindly supplied by S. Razin (pMC5), T. Samuelsson (pMM22, pMM25, pTZ19Rgly, pTZ19Rleu, and m13mp9), C. Christiansen (pMYC405, pMYC708, and pMYC714), and A. Muto (pMCB1088). References describing their construction are shown in parentheses.

direction of the map for *M. mycoides* subsp. *mycoides* Y goat corresponded to the clockwise direction in the circular map (10). For each map, the *Bgl*I fragment placed to the right of the zero coordinate was the one reacting most strongly with the pTZ19Rleu probe.

Comparison of the genomic maps for the four SC *M. mycoides* subsp. *mycoides* strains showed all mapped restriction sites conserved. Similarly, relative positions for all the mapped loci were conserved, as well as the orientation of the weak and strong reactions of the pMC5 probe with the fragments adjacent to the *Sma*I sites in the *rrn* loci. Thus, the only differences found among the genomes lay in their total sizes and in the distances between some of the restriction sites.

Comparison of the maps of the SC strains with that of strain Y goat suggested considerable conservation of genome structure. All of the gene loci mapped, except one, were similarly placed, and the orientation of intensity of response to probing around the *rrn Sma*I sites was the same. The exception was *rpoC*. For the strain Y goat genome, this was detected, together with *atp* and *glyA*, in the 78-kbp *Sal*I B fragment with *trnA* at one end. In the SC genomes, the loci *atp*, *glyA*, and *trnA* were similarly close together, within the overlap of *Sal*I C and *Xho*I B, but the *rpoC* locus was removed from them by at least 140 kbp to reside in the overlap of *Sal*I A and *Bgl*I C. Proceeding from the left-hand *rrn* locus to just beyond the *trnA* locus, the order of mapped restriction sites in the region around *rpoC* was *Sma*I, *Bgl*I, (*Xho*I, *Bgl*I, *Bgl*I*, *Sal*I, *rpoC*), *Sal*I, and *Bgl*I for Y goat and *Sma*I, *Bgl*I, (*rpoC*, *Sal*I, *Bgl*I, *Xho*I), *Sal*I, and *Bgl*I for the SC genomes. These sequences could be related by an inversion of one of the regions in parentheses and loss of the *Bgl*I* site.

Comparison of the map for the PG50 genome with those for the LC and SC strains showed it to be appreciably smaller, but with *rrn* operons in a similar relative position and again showing the same orientation of intensity of response to the pMC5 probe. The *atp* and *rpoC* loci were close together within the overlap of *Bgl*I D and *Sal*I A, and it is known from isolation of the pMYC714 clone (12) that the *gly*A gene lies between these two loci in the PG50 genome, corresponding to the close association of these three loci in the genome of strain Y goat. However, the *trnA* locus of PG50 was not close to the *atp* and *glyA* loci between the two *rrn* loci as it was in the genomes of the *M. mycoides* strains; instead, together with the *trn*C and *rpn* loci, it lay distal to *leuT*.

Comparison of genomic maps may have significance in relation to taxonomy, phylogeny, and the question of conservation and functional importance of gene order in procaryotic genomes. With regard to taxonomy, the four strains classified as SC *M. mycoides* subsp. *mycoides* showed differences only in the total sizes of their genomes and the distances between some restriction sites. Their maps showed considerable similarity to that for the LC strain, Y goat, but differed in the placement of the *rpoC* locus as well as in the number and placement of some restriction sites. As noted above, another LC strain, GC1176-2, shows congruence with Y goat when three regions of its larger genome are looped out from the superposition (19a). This congruence applies to all the loci mapped here plus several others (J. C. Whitley and L. R. Finch, unpublished data). The PG50 map was different from either the LC or SC maps, although aspects of its gene placement showed clearly identifiable relationships to the *M. mycoides* strains. Thus the taxonomic criterion provided by a comparison of gene placement on genomic maps gave conclusions consistent with the existing classification of these closely related organisms.

Considering phylogeny, the maps suggested that the SC strains could be related to the Y goat strain by an inversion of a >140-kbp region encompassing *rpoC*. The relationship of PG50 to Y goat could involve a translocation and inversion of a region containing *trnA*, *trnC*, and *rpn*, or perhaps at least two independent inversions. Starting with the Y goat genome, one of these could have separated *trnA*, *trnC*, and *rpn* from the adjacent *rrn* locus and placed them inverted and distal to *leuT*. The second would have encompassed the second *rrn* operon and *atp*, *glyA*, and *rpoC* and restored their original orientation while separating the latter three loci from *trnA*.

The conservation of gene order between *Escherichia coli* and *Salmonella typhimurium* has been considered by Riley and Krawlec (14) to imply a functional correlate of the gene order that confers a growth or survival value to the organism. They note that a number of loci which are close to the origin of replication in these genomes are similarly placed in the *Bacillus subtilis* genome. A broad region containing the origin of replication has been outlined for *M. mycoides* subsp. *mycoides* Y goat (10). It is most probably in the region (*Bam*HI E restriction fragment) covering and just to the left of the *atp*, *glyA*, and *rpoC* loci on the Y goat map. This placement is consistent with a functional conservation, since the *atp* and *rpoC* loci are within a few minutes to either side of the *oriC* locus in *E. coli* and *S. typhimurium*. Although *gly*A is well removed, several loci related to methionine biosynthesis are close to *oriC* (2, 19). In *M. mycoides* subsp. *mycoides* Y goat, which does not effect complete methionine biosynthesis but methylates DNA from

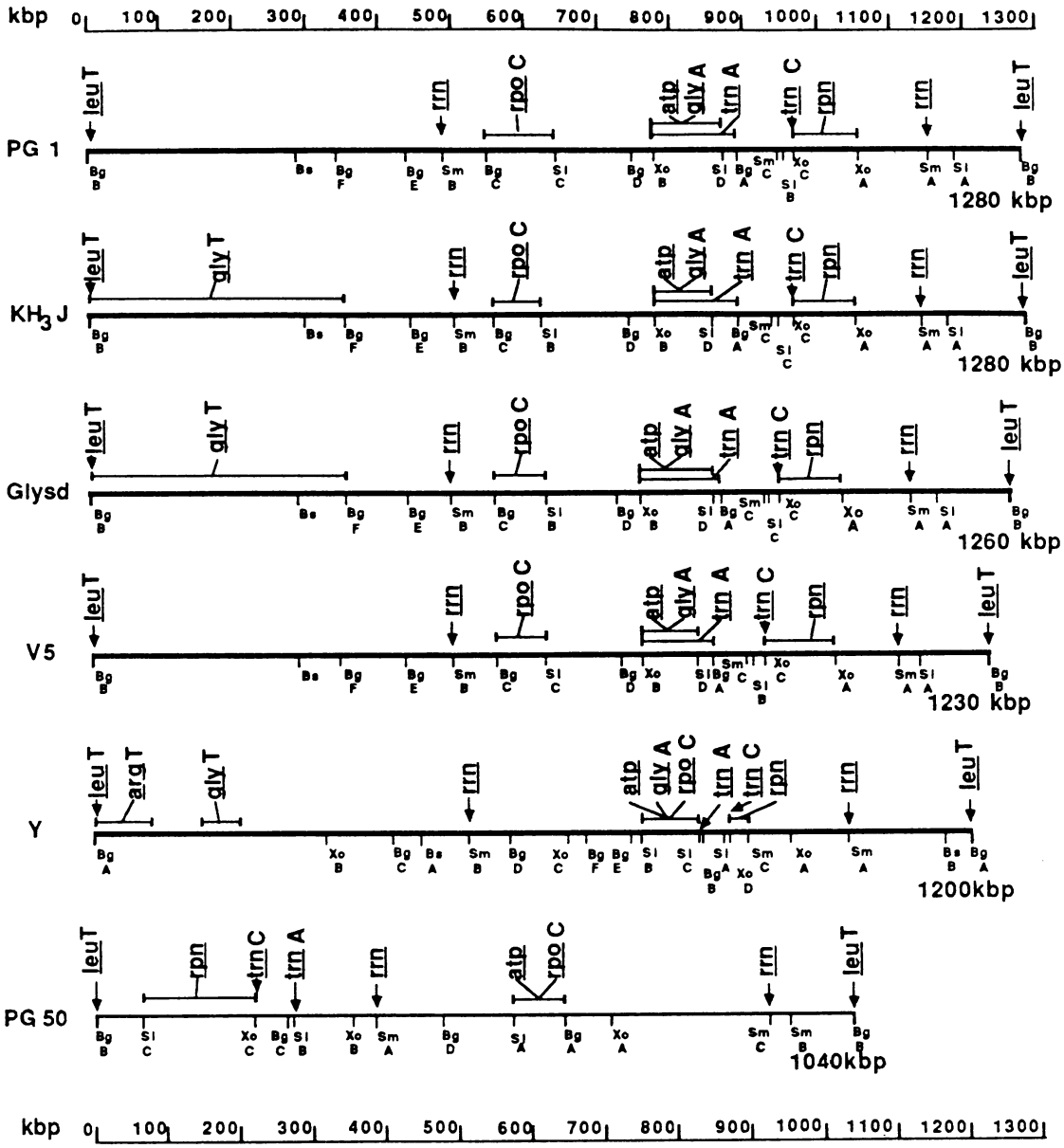

FIG. 1. Physical genomic maps for strains of SC and LC *M. mycoides* subsp. *mycoides* and *Mycoplasma* strain PG50. Maps were constructed from data with restriction fragments, including their reaction to the probes defined in Table 1. For presentation, each of the circular genomes is shown linearized from a *BglI* site between the two *BglI* fragments which responded to a probe containing DNA coding for a $tRNA^{Leu}$. This site is shown at the initial (zero) and final coordinates, with the more strongly reacting *BglI* fragment placed to its right. Abbreviations: Bg, Sl, Sm, and Xo, cleavage sites for the restriction endonucleases *BglI*, *SalI*, *SmaI*, and *XhoI*, respectively; Glysd, Gladysdale strain. The subscripts, or suffices, A, B, C, etc., to these abbreviations designate the single digest restriction fragment which is shown to the right of the cleavage site, e.g., *BglI* A is to the right of BgA. Symbols identifying the loci are defined in Table 1. Symbols: ▼, probe hybridized with fragments on both sides of the indicated cleavage site; ⊢——⊣, hybridization with the probe was confined to sequences somewhere within the restriction fragment spanned by the horizontal line.

methionine (2a), the 1-C units for remethylation of homocysteine to methionine could derive from N^5,N^{10}-methylene tetrahydrofolate produced together with glycine in the reaction catalyzed by the product of the *glyA* gene. Tetrahydrofolate metabolism has been noted in *M. mycoides* subsp. *mycoides* Y goat (7). Further comparison of gene placement on physical genomic maps will add to knowledge of the conservation of gene order and its physiological significance.

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