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Rapid physical mapping of the *Mycoplasma mobile* genome by two-dimensional field inversion gel electrophoresis techniques

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ABSTRACT

A macrorestriction map of the 780 kbp *Mycoplasma mobile* (ATCC 43663) genome was constructed. Linking fragments were identified on two-dimensional pulsed-field electrophoresis gels. Either complete double restriction digests or partial and complete single digests were separated in the first and second dimension, respectively. 19 restriction sites of four enzymes could be assigned to the map. These rapid methods do not require DNA probes and are applicable to the long-range restriction mapping of all genomes that yield resolvable patterns on two-dimensional gels.

INTRODUCTION

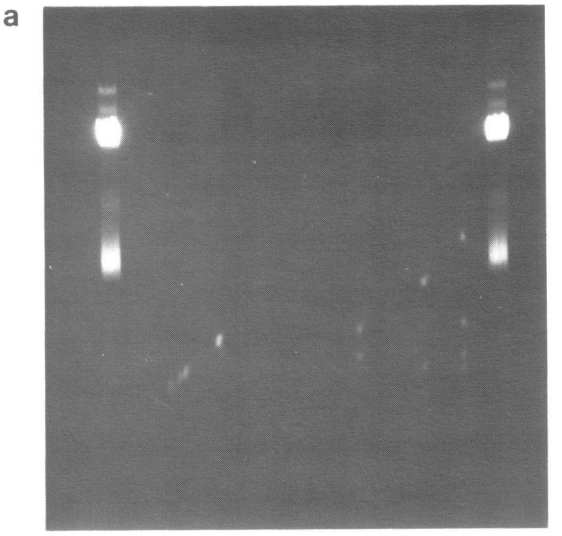
Mycoplasmas are the simplest self-replicating cellular organisms with the smallest recorded genome sizes. Genetic analysis of these organisms is hampered by difficulties in isolation of stable mutants and the lack of appropriate gene transfer systems. But since the total genome size is in the resolution range of pulsed-field gel electrophoresis (PFGE) (1) techniques, such as field inversion gel electrophoresis (FIGE) (2), this should allow complete physical mapping of the genome. The power of these techniques has recently been demonstrated by the publication of physical maps of the *Escherichia coli* K12 (3) and *Mycoplasma mycoides* subsp. mycoides Y (4) genomes. The work described in the present paper deals with the construction of a genomic macrorestriction map of *Mycoplasma mobile* (ATCC 43663) (5) using a novel two-dimensional field inversion gel electrophoresis (2D-FIGE) approach (6). This method allows construction of a complete macrorestriction map in a few weeks without using any hybridisation.

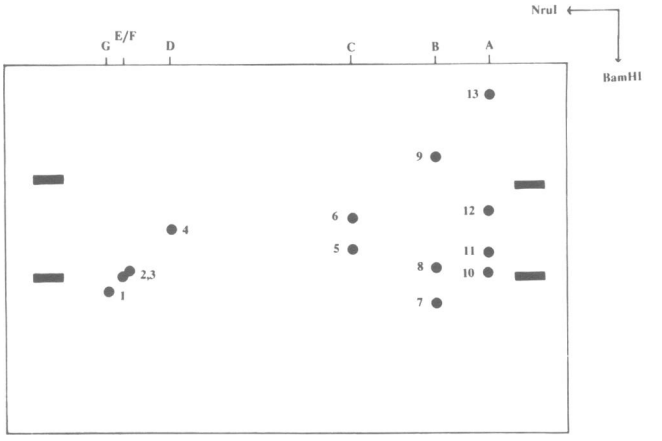
MATERIALS AND METHODS

M. mobile was grown in Modified Hayflick's Medium (7) for 48 h at 25°C, pelleted and resuspended in PBS (1.44 g $Na_2HPO_4*2H_2O$, 0.3 g KH_2PO_4, 0.2 g KCl and 8.0 g NaCl per liter) at about 3×10^9 c.f.u./ml. Different dilutions were incorporated in low gelling agarose (Sigma). Since the mobility of DNA in a FIGE run is reduced in the high DNA concentration range (8), the minimum DNA concentration which still allows clear visualization of all bands was empirically determined for each experiment to ensure concentration independence of the fragment mobility. It is given in the respective figure legends. Preparation of chromosomal DNA and restriction endonuclease digestion for the one- and two-dimensional FIGE were performed

Table I. Fragment sizes in kbp of *M. mobile* DNA digested with *ApaI, MluI, BamHI* and *NruI*, respectively. The fragment sizes given in brackets are those used for the construction of the map.

Enzyme	ApaI		MluI		BamHI		NruI	
Fragment	ApA	(705)	Ml A	355 (355)	Ba A	230 (230)	Nr A	295 (285)
Size	Ap B	75 (75)	Ml B	320 (320)	Ba B	190 (190)	Nr B	165 (165)
(kbp)			Ml C	105 (105)	Ba C	175 (175)	Nr C	135 (135)
					Ba D	80 (80)	Nr D	75 (75)
					Ba E	55 (55)	Nr E/F	45 (45)
					Ba F	50 (50)	Nr E/F	45 (45)
							Nr G	35 (30)
Sum (kbp)		(780)		780 (780)		780 (780)		795 (780)





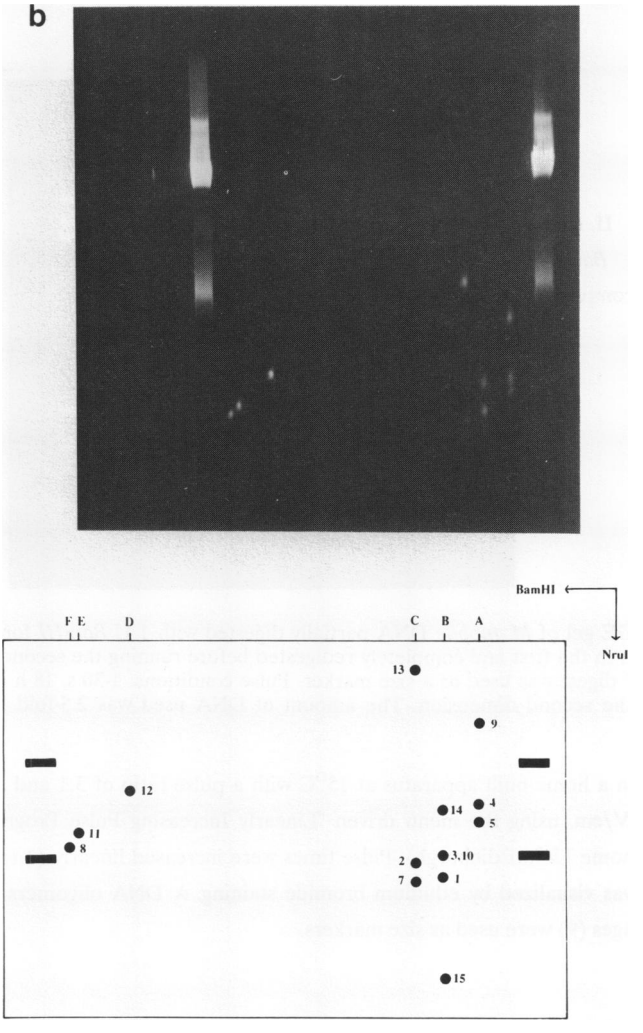


Figure 1: 2D-FIGE gel of *Mycoplasma mobile* DNA, digested first with *NruI* and then *BamHI* (a) and with *BamHI* first and then *NruI* (b). λgT10 oligomers (monomer size 43.34 kbp) were used as size markers. Identical DNA spots on both gels are marked with the same number in the accompanying diagrams. The two black bars indicate the position of the λ mono- and dimer. Fragment 6 in (a) contains the hard-to-cut *NruI* site (see text) which has been cut in (b) yielding fragments 14 and 15. Pulse conditions: 1-10 s, 18 h for *BamHI* and 1-15 s, 18 h for *NruI* in the first dimension, and 1-10 s, 18 h in the second. Agarose blocks with a DNA content equivalent to about 8×10^6 c.f.u. were used in the first dimension.

as described previously (6,9). All double digestions were performed sequentially. *ApaI* required a somewhat higher concentration of about 1U/μl restriction buffer to achieve complete cleavage (as compared to the 0.2 U/μl normally used). For the second digestion in a 2D-FIGE run, about 75 U of enzyme per ml restriction buffer were used.

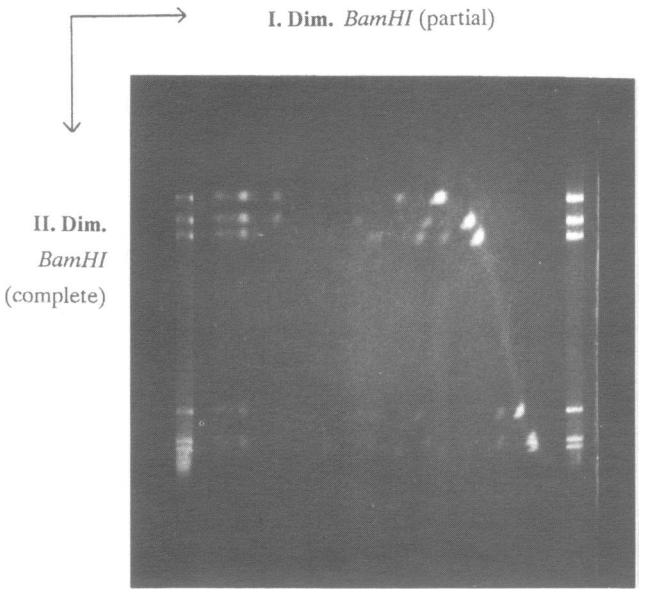


Figure 2: 2D-FIGE gel of *M. mobile* DNA partially digested with 1 U *BamHI* for 1.5 h at 37°C before separation in the first and completely redigested before running the second dimension. A complete *BamHI* digest was used as a size marker. Pulse conditions: 1-30 s, 18 h in the first and 1-10 s, 18 h in the second dimension. The amount of DNA used was 2.5-fold higher than in figure 1.

FIGE was run on a home-built apparatus at 15°C with a pulse ratio of 3:1 and an electric field strength of 5.6 V/cm, using the menu driven "Linearly Increasing Pulse Program" (B.Gibson, Mammalian Genome Unit, Edinburgh). Pulse times were increased linearly (1 ramp). After the run, the DNA was visualized by ethidium bromide staining. λ DNA oligomers prepared from intact λgT10 phages (9) were used as size markers.

RESULTS

Sizes of fragments

The sizes of fragments from the digestion of *M. mobile* DNA with *ApaI, MluI, BamHI* and *NruI* are displayed in table I. They represent the best estimates from several FIGE runs at different pulse times and are given as multiples of 5 kbp. With a 3- to 5-fold overdigestion, *NruI* revealed an additional cleavage site in the middle of fragment Nr **C**, yielding Nr $\mathbf{C_a}$ and Nr $\mathbf{C_b}$.

The total genome size was estimated to be 780 ± 30 kbp. This value was used for the construction of the map.

Construction of a macrorestriction map.

Method A: Identification of linking fragments by a matched restriction enzyme pair analysis. The genomic DNA is digested with two different restriction endonucleases and the fragments are

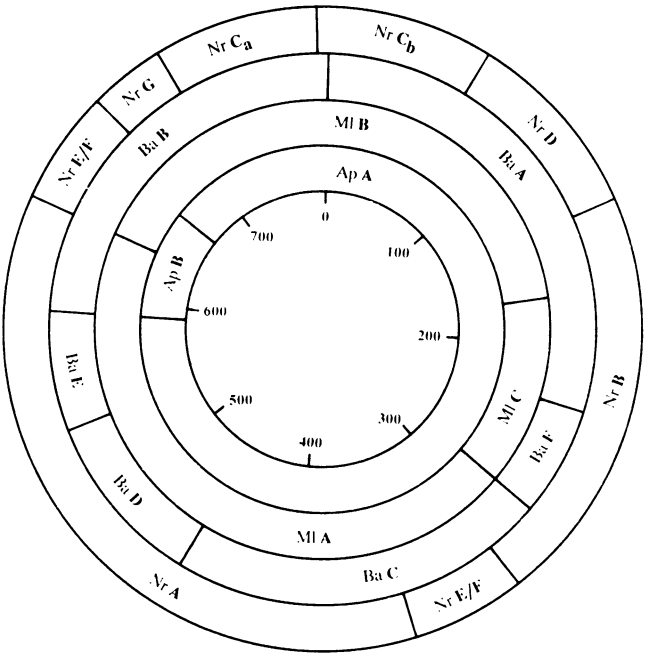


Figure 3: Macrorestriction map of *Mycoplasma mobile*, ATCC 43663. The *BamHI* site between fragments Ba **A** and Ba **B** was arbitrarily placed at 0 kbp. For fragment symbols see table I.

separated before and after the second digestion by FIGE. By combining two complementary 2D-FIGE gels with reverse order of the two digestions, it is possible to elucidate the sequence of the restriction fragments: Identical DNA spots on both 2D-FIGE gels are identified and assigned to the respective fragments of the first dimension which must therefore overlap on the map. Figure 1 displays the 2D-FIGE gels obtained for *M. mobile* DNA double digested with *BamHI* and *NruI*. Fragment 13, for instance, is a double digestion product of the fragments Nr **A** and Ba **C** (Fig.1a,b). These two fragments must therefore overlap on the map. Furthermore, since fragment 12 is identical with fragment Ba **D** because it has not been cleaved by *NruI* in the second dimension, it must be part of a *NruI* fragment with two or more *BamHI* cleavage sites. Since fragment Nr **B** does not contain a double digestion fragment of the appropriate size (Fig.1a), fragment Ba **D** must be part of fragment Nr **A** and is, therefore, located close to Ba **C** on the *M. mobile* chromosome. In this way, most of the fragments can unambiguously be located on the map. However, ambiguities arise with inaccuracies in molecular weight determination and more than two restriction sites per fragment in the second dimension. E.g., fragment Nr **A** contains both *BamHI* fragments Ba **D** and Ba **E**, such that their order with respect to fragment Ba **C** is inherently indeterminate. However, this ambiguity could be resolved by the partial digest analysis described below.

Method B: Restriction fragment analysis of partial digests. A partial digestion of the genomic DNA with one enzyme is separated by FIGE followed by redigestion to completion with the same enzyme and separation by FIGE in the second dimension. In this case, the sequence of the restriction fragments can directly be read off from a single gel. The respective 2D gel of *M. mobile* DNA digested with *BamHI* is shown in figure 2. All six possible linked *BamHI* fragment pairs can be identified on the print. From this gel pattern the *BamHI* fragment order Ba **A-F-C-D-E-B** can be derived.

The map positions of the *MluI* and *ApaI* restriction sites were determined by conventional single and double restriction endonuclease digestion of the genomic DNA and separation of the fragments by one-dimensional FIGE (data not shown). The order of the *NruI* fragments Nr **E/F** and Nr **G** (Fig.3) is revealed by the *ApaI* site in fragment Nr **E/F**.

The genome map of *M. mobile* derived from these experiments is shown in figure 3. At small pulse times, the two *NruI* fragments, designated Nr **E/F** (table I), display a small size difference of approximately 1 kbp which is within the limits of error of the molecular weight determination by FIGE and, therefore, not represented in the map. However, the 2D-FIGE gels in figure 1 suggest that the larger one of these two fragments is part of fragment Ba **B**.

The map allows assignement of any cloned DNA probe to a map interval of 15% or less of the total genome size.

DISCUSSION

The apparent genome size of about 780 kbp for *Mycoplasma mobile* is in good agreement with previous data for several other *Mycoplasma* species determined by renaturation kinetics (10) and electron microscopy studies (11) but in contrast to recent genome size estimates by PFGE (12). The reason for this discrepancy is not clear. The higher DNA concentration and the different size standard used in the PFGE experiments by Pyle et al. (12) may in part explain this difference. In addition, it has to be considered that considerable genome size differences between different members of the same family may exist, although in one case, at least, the same species (*M. gallisepticum* PG31) has been examined by renaturation kinetics (10) as well as PFGE (12).

The 2D-FIGE methods presented here are applicable to all genomes of prokaryotes and lower eukaryotes for which appropriate rare-cutting endonucleases can be found. These latter are selected according to the length of their recognition sequence and the GC-content and oligonucleotide frequency of the genomic DNA (13). With larger genomes and a correspondingly higher number of fragments sensitivity problems may arise with ethidium bromide staining, in particular in the low molecular weight range. In this case, it is advisable to concentrate the DNA bands after the first dimension (14) and to visualize all spots of the 2D gel by Southern blot analysis with labelled total genomic DNA. For more complex genomes, a computer-aided analysis of 2D-FIGE gels of several pairs of enzymes may allow construction of a map via reciprocal linkage exclusion. In fact, the whole set of strategies established for cosmid mapping (15) may be utilized if suitably adapted to PFGE conditions.

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