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A physical map of the genome of *Ureaplasma urealyticum* 960^T with ribosomal RNA loci

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ABSTRACT

A physical map is presented for the 900 kilobase pair genome of *Ureaplasma urealyticum* 960^T, locating 29 sites for 6 restriction endonucleases. The large restriction fragments were separated and sized by pulsed-field agarose gel electrophoresis (PFGE). Their locations on the map were determined by probing Southern blots of digests with individual fragments isolated from other digests and by correlating the products of double digestions and partial digestions. An end-labelling technique was used to detect small fragments not readily observed by PFGE. Two loci for rRNA genes have been determined by probing with cloned DNA.

INTRODUCTION

The development of techniques using pulsed-field gel electrophoresis (PFGE) for the construction of physical maps of procaryote genomes, first applied to *Escherichia coli* (1), has now given maps for two species of mycoplasma, *Mycoplasma mycoides* subsp. *mycoides*, strains Y (2) and GC1176-2 (3), and *Mycoplasma mobilis* (4). A genomic map for another species, *Mycoplasma pneumoniae*, has been substantially completed by a cosmid mapping technique (5). As more such maps become available, they should be valuable aids to assessing phylogeny and taxonomy amongst the *Mollicutes*, to which the mycoplasmas belong. The genomes for the mycoplasmas noted above differ considerably in size, being mapped as 1200 and 1380 kilobase pairs (kbp) respectively for the two *M mycoides* strains and 780 and approximately 800 kbp for *M. mobilis* and *M. pneumoniae*, respectively. *Ureaplasma urealyticum*, a human pathogen, is a member of a second genus of the *Mycoplasmataceae*. Its genome, reported to be amongst the smallest of procaryote genomes (6), has been estimated at approximately 900 kb, for *U. urealyticum* 960^T, by PFGE (7). The ureaplasmas are characterized by an ability to hydrolyze urea, possibly as a major energy source (8). We have constructed a genomic restriction map for *U. urealyticum* 960^T and determined rRNA loci (*rrn*) on it to provide data towards a comparison of genome structure in mollicutes and to commence a genetic study of this interesting organism.

MATERIALS AND METHODS

U. urealyticum 960T (ATCC27618) was grown, harvested and washed as reported previously (9). The cells were incorporated

Table 1. Data on Restriction Fragments from the DNA of *U. urealyticum* 960[T]

Enzyme	Fragment	Size (kb) Meas.	Map	Response to probes	Products of digestion[a]
*Apa*I	ApA	903[b]	900	all probes reacted	As[C21,A,D,F,G,B,H,E,C36], Bg[C21,D,B,E,A,F,C178], Bs[C20,B,F,A,D,E,C122], Sm[A538,A365], Xo[B188,D,E,F,A,C,B72]
	Total	903	900		
*Asp*718	AsA	607	604	BsA, BsB, XoA, pMC5 pMM25	Bg[D,B,E,A295],Bs[B,F,A338], Sm[A337,A274], Xo[B170,D,E,F,A352]
	AsB	102	95	BsC,BsE, BgC, pMC5	Bg[F,C95],Bs[E,C35], Xo[A30,C73]
	AsC	58	58	BsC, BgC, XoB	Ap[A36,A21]
	AsD	56	56	BsD	Bs[D40,A18]
	AsE	50	49	BsC, BgC, XoB	Xo[C12,B35]
	AsF	18	18		
	AsG	18	18		
	AsH	2	2		
	Total	911	900		
*Bgl*I	BgA	388	376	BsA, BsD, XoA, pMC5, pMM25	As[A295,D,F,G],Bs[A325,D], Sm[A17,A345]
	[c]BgB	218	224	BgB, BsB, XoB, XoD	Bs[A35,F,B164], Xo[B85,D,E,F,A32]
	BgC	198	200	BgC, BsC, BsE, XoB, pMC5	Ap[A178,A21],As[B95,H,E,C], Bs[E65,C],Xo[A30,C,B95]
	BgD	68	69	BgD, BsB, XoB	
	BgE	25	23		
	BgF	4	4	BsE, pMC5	
	BgG	4	4	pMC5	
	Total	905	900		
*Bss*HII	BsA	352	357	BsA, pMM25	As[A338,D18],Bg[B35,BgE,A325], Sm[A78,A285]
	BsB	245	244	BsB, XoB, XoD, BgB, BgD, pMC5	Bg[F,D,B164],Xo[B170,D,E]
	BsC	138	138	BsC, XoB, XoC	Ap[A122,A20], As[B35,H,E,C],Xo(C50,B95]
	BsD	74	74	BsD	As[D40,F,G]
	BsE	66	67	BsE, pMC5	Xo[A30,C38],Bg[F,C65]
	BsF	21	20	BgB	Xo[F,A15]
	Total	896	900		
*Sma*I	SmA	903[b]	900	all probes reacted	Ap[A538,A365], As[A274,D,F,G,B,H,E,C,A337], Bg[A345,D,E,C,B,F,A17], Bs[A285,D,E,C,B,F,A78], Xo[A390,C,B,D,E,A90]
	Total	903	900		

Table 1 (continued)

Enzyme	Fragment	Size (kb) Meas.	Map	Response to probes	Products of digestion[a]
*Xho*I	XoA	470	475	pMC5	As[A352,D,F,G,B30], Bg[B32,E,A,F,C30], Bs[F15,A,D,E30],Sm[A90,A390]
	XoB	256	253	pMC5	Ap[A72,A188],As[E35,C,A170], Bg[C95,D,B85],Bs[C95,B170]
	XoC	81	81		As[B73,H,E12],Bs[E38,C50]
	XoD	54	54		
	XoE	33	33		
	XoF	7	7		
	Total	901	900		

[a]The product fragments are shown in clockwise order around the genome as mapped in Figure 1. Thus as an example, the representation As[C21,A,D,F,G,B,H,E,C36] in the first line indicates that the ApA fragment was cleaved by *Asp*718 to give 21 kb from the clockwise end of AsC, plus AsA, AsD, AsF, AsG, AsB, AsH and AsE, plus 36 kb from the anti-clockwise end of AsC.
[b]Based on the sum of the sizes of the *ApaI*/*Sma*I double digestion products.
[c]BgB is slowly digested by *Bgl*I to fragments of 49 and 175 kb.

into blocks of agarose (FMC Sea Plaque) for *in situ* release of their DNA and its subsequent treatment by restriction endonuclease digestion and field inversion gel electrophoresis (FIGE) to separate the restriction fragments for genomic restriction mapping as described by Pyle and Finch (2) for *M. mycoides* subsp. *mycoides* Y. Fragment sizes were measured by comparison with digests of *M. mycoides* DNA (1, 7) and/or λ DNA multimers and restriction digests. The sizes for the *M. mycoides* DNA restriction fragments used as markers were measured against and consistent with those of the λ markers. Ordering of contiguous fragments which were not cut in any of the digests with other restriction enzymes was achieved by probing and sizing of partial digestion products. Some of the partial digestions were performed on individual fragments from digests with other endonucleases, using the appropriate band excised from the gels (2). Previously described (2) methods were also used in probing with the plasmids pMC5 (10), kindly made available by Professor S. Razin, and pMM25 (11), kindly supplied by Professor T. Samuelsson, to detect restriction fragments containing loci for rRNA genes (rrn) and a tRNA gene cluster, respectively.

A more sensitive technique was implemented to detect small (<10kbp) restriction fragments which might be lost from, or have insufficient intensity of ethidium bromide staining to be detected on PFGE. The DNA fragments from single or double digests were labelled in the agarose block by incubation for 30 min at room temperature in a buffer solution containing 0.2 M HEPES, pH 6.6, 0.01 M $MgCl_2$, 1 mM dithiothreitol, 1 μCi [^{32}P]dATP or [^{32}P]dCTP (BRESA), other deoxynucleoside triphosphates unlabelled at 0.05 mM and 1 unit of Klenow DNA polymerase (Boehringer-Mannheim). The reaction was stopped by removal of the buffer solution and addition of 10 mM Tris/HCl, 1 mM EDTA, pH 8.0 (TE). After leaving at room temperature for 30 min, the block was washed once with TE to remove residual free nucleotides and then sealed with low melting point agarose into a well of a 1% agarose gel for separation of small restriction fragments by direct

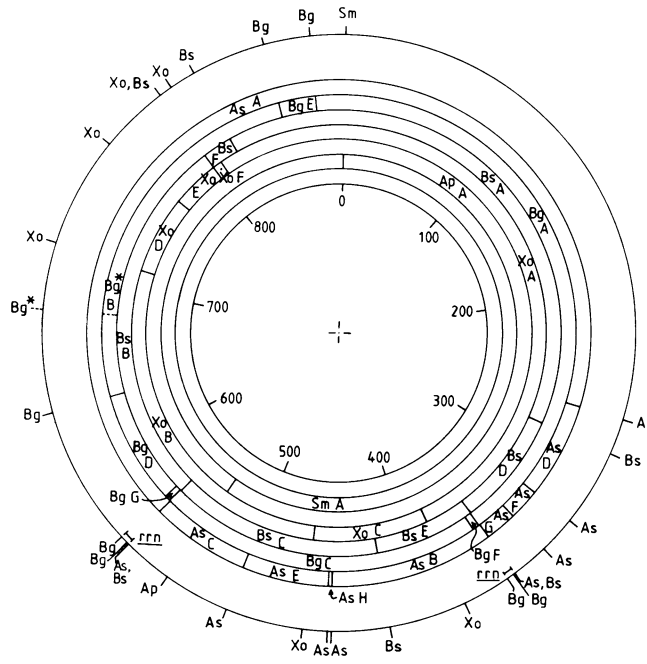

Figure 1. Restriction endonuclease map of the genome of *U. urealyticum* 960. The map was constructed from the data on restriction fragments presented in Table 1., rounded to a total genome size of 900 kbp, together with information from partial digestions. The abbreviations Ap, As, Bg, Bs, Sm and Xo are used for the restriction endonucleases ApaI, Asp718, BglI, BssHII, SmaI and XhoI, respectively, to mark their cleavage sites around the outer circle or to identify the segments corresponding to particular restriction fragments within the inner rings. The sites marked *rrn* within the outermost ring indicate the loci identified by probing with pMC5. The inner circle shows distances clockwise around the genome in kbp starting from the single SmaI site.

electrophoresis at 0.8 V/cm for 16 to 20 h. Similarly labelled digests of λ DNA were run as markers. The gels were dried down and autoradiographed to detect the presence of bands smaller than 15-20 kbp.

RESULTS

The data obtained on the restriction digests of the *U. urealyticum* genome are presented in Table 1 and used to plot the restriction map of the genome shown in Fig. 1. Construction of the map followed the rationales used for the *M. mycoides* genomic restriction map (2). Restriction sites were positioned on the map after subjective correlation of all the data on fragment sizes in single and double digests. Genomic size was rounded to 900 kbp from the range of 896 to 911 kbp for the sums of the measured

sizes of the fragments in the individual single digests (Table 1).

Gels of some BglI digests showed extra bands corresponding to fragment sizes of 49 and 175 kbp. Probing and double digestions showed that the extra fragments derived from a partial digestion of BgB at the site indicated by Bg* at about 690 kbp on the map in Fig. 1. The limitation on the extent of digestion at this site was kinetic, with the cleavage approaching completion with more vigorous digestion.

The responses (Table 1) of restriction fragments to probing for detection of the loci for rRNA operons (*rrn*) indicate the loci at the sets of AspI, BssHII and BglI sites near 360 and 560 kbp. Only the strongly responding fragments are indicated in Table 1. The other Asp718, BglI and BssHII fragments abutting these sites gave weak but possibly significant responses. Initially, the only evidence on the two small BglI fragments (BgG and F) was the strong response to probing by a band of high mobility with negligible fluorescent staining. To establish whether small BglI fragment(s) were associated with one, or both *rrn* operons, BglI digests were performed on excised fragment bands from digests by other endonucleases. Small fragments from the second digestion were then detected by labelling as described in Materials and Methods. These experiments showed that small (3.7 kbp) BglI fragments were generated from XoA (BgF) and from XoB and AsA (BgG). Complete digests with Asp718 or BssHII, partially digested with BglI, showed bands corresponding to 3.7 and 4.0 kbp. This suggested that Asp718 and BssHII sites were approximately 0.3 kbp outside the 3.7 kbp BglI fragments. However, no band with a mobility corresponding to a size of 0.3 kbp was detected from any digest. Possibly such smaller fragments might not be detected because of their less intense labelling and/or because of loss from the agarose block during the digestion and labelling procedures. Similarly, no very small fragment corresponding to an Asp718/BssHII digestion product was detected. However, a band corresponding to a size of 1.6 kbp (AsH) was found in the Asp718 digests and subsequently shown to lie in BgC and XoC by Asp718 digestion of the excised bands followed by the end-labelling procedure. Similar examination of all single digests and some double digests identified only one further small single digestion product (XoF, 6.7 kbp), shown to lie in, or almost entirely in, BsF; and one double digestion product from the 12 kbp overlap of AsE and XoC.

DISCUSSION

Fig. 1 presents a physical map for the genome of *U. urealyticum* 960^T showing 29 restriction sites and the loci for the two rRNA operons. The map is consistent with the approximate estimate of 900 kbp for the size of the *U. urealyticum* 960^T genome made previously by PFGE (7).

The data in Table 1 on responses to pMM25, which contains sequences for a cluster of 9 tRNA genes (11), indicates that homology to all or some of the genes lay within the 295 kbp overlap between AsA and BgA. The data do not establish whether the response to probing might be constrained to a small region of the genome, consistent with the occurrence of a clustering of the 9 tRNA genes in *U. urealyticum*, or whether it might be dispersed, along with the component tRNA genes, within the 295 kbp region defined by probing.

The slow digestion of the BglI site at approximately 690 kbp in Fig. 1 might be explicable by methylation at 5'-GCNGC-3' sites in *U. urealyticum* 960^T DNA. The strain possesses a restriction endonuclease, Uur960I which recognises this sequence and its DNA is not cut by the enzyme or the isoschizomeric Fnu4HI (9), suggesting that the recognition site should have cytosine methylation. It is known that BglI is inhibited by full methylation on the 3'-cytosines in both strands of its recognition sequence, 5'-GCCNNNNNGGC-3' and partially inhibited by hemimethylation (12). Methylation of one cytosine on each strand of a Uur960I site having an appropriate overlap with a BglI site would cause the latter to be hemimethylated and thus of lowered reactivity as observed for the site noted above.

The technique of labelling fragments in agarose followed by separation through direct agarose gel electrophoresis provides greater sensitivity for detection of small fragments present at the low copy numbers used in the manipulations of genomic DNA in agarose. Theoretically, the procedure involved an end-labelling reaction so that the intensity of bands on autoradiographs should be independent of fragment size. This was largely true for λ DNA fragments, but not with mycoplasma DNA for which observations suggest internal, and therefore potentially greater, labelling of the fragments. The labelling could also be used to detect larger fragments when their low copy number impedes detection by ethidium bromide staining after PFGE separation. Its application might be advantageous to the analysis of restriction digests of small eukaryotic chromosomes isolated and digested as excised bands from PFGE.

It is of interest to compare the map of the *U. urealyticum* genome with the others published to date. For the genome of *M. mycoides* subsp. *mycoides* Y, it was noted (2) that one of the two regions bounded by the two rRNA operons was much richer in mapped restriction sites (G+C rich) than the other. A similar pattern was observed for *M. mycoides* subsp. *mycoides* GC1176-2 (3). For *U. urealyticum* 960^T, 8 of the mapped sites appear to be within, or closely associated with the *rrn* loci. If these are excluded, the distribution of the remaining sites appears fairly even over approximately 630 kbp of the genome but there are no sites in the remaining 270 kbp between the SmaI site and the first Asp718 site clockwise from it. It was speculated previously (2) whether uneven distribution of G+C sites might be indicative of the functional significance of the regions in which they are situated. With the other mycoplasma genomes mapped, that for *M. pneumoniae* has been presented as a set of overlapping cosmids rather than as a restriction map and for *M. mobilis* (4) a smaller number of restriction sites has been determined. The small number of sites and the absence of any *rrn* locus on the latter map makes documentation of an unevenness in distribution of the sites less certain. The largest NruI fragment, comprising 285 kbp of the 780 kbp genome, contains only 4 of the 18 sites mapped but this hint of a scarcity of (G+C) rich sites in the fragment could easily prove misleading after the mapping of a few more sites. It would be of interest to determine whether differences in the distribution of (G+C) rich sites correlate with differences in the overall base composition of the regions involved.

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REFERENCES

1. Smith, C.L., Econome, J.G., Schutt, A., Klco, S. and Cantor, C. (1987) Science 236:1448-1453
2. Pyle, L.E. and Finch, L.R. (1988) Nucleic Acids Res. 16:6027-6039
3. Whitley, J.C., Bergemann, A.D., Pyle, L.E., Cocks, B.G., Youil, R. and Finch, L. R. (1989) Zbl. Bakt. Mikrobiol. Hyg. Supp. Vol. (in press).
4. Bautsch, W. (1988) Nucleic Acids Res. 16:11461-11468
5. Wenzel, R. and Herrmann, R. (1988) Nucleic Acids Res. 16:8323-8336.
6. Razin, S., Barile, M.F., Harasawa, R., Amikam, D. and Glaser, G. (1983) Yale J. Biol. Med. 56:357-366
7. Pyle, L.E., Corcoran, L.N., Cocks, B.G., Bergemann, A.D., Whitley, J.C. and Finch, L.R. (1988) Nucleic Acids Res. 16:6015-6025.
8. Romano, N., Tolone, G., Ajello, F. and LaLicata, R. (1980) J. Bacteriol. 144:830-832.
9. Cocks, B.G. and Finch, L. R. (1987) Int. J. Syst. Bacteriol. 37:451-453.
10. Amikam, D., Razin, S. and Glaser, G. (1982) Nucleic Acids Res. 10:4215-4222.
11. Samuelsson, T., Elias, P. Lustig, and Guindy, Y.S. (1985) Biochem. J. 232:223-228.
12. Nelson, M. and McClelland, M. (1987) Nucleic Acids Res. 15:1219-1230.