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Mycoplasma Gallisepticum strain S6 genome contains three regions hybridizing with 16 S rRNA and two regions hybridizing with 23 S and 5 S rRNA

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Southern hybridization and cloning experiments revealed existence of 3 regions hybridizing with 16 S rRNA and 2 regions hybridizing with 23 S and 5 S rRNA in Mycoplasma Gallisepticum strain S6 genome thus forming 4 separate contiguous regions. One set of a putative rRNA genes is organized classically for eubacteria order 16 S–23 S–5 S. The other two 16 S rRNA and the other one 23 S–5 S rRNA hybridizing regions are separated from each other and from the complete rRNA operon for a distance of more than 6 kb.

rRNA; Genome; Mycoplasma Gallisepticum

1. INTRODUCTION

The hybridization of digested DNA of one procaryotic organism with labeled DNA from rRNA genes of another organism has become a powerful tool for studying the rRNA genes in different bacterial strains. These experiments revealed the presence of low copy number of rRNA genes in genome of Mycoplasmas, as well as the existence of split rRNA operons in Mycoplasmas [1–3]. Nevertheless, to our knowledge the number of regions hybridizing with either 16 S rRNA, 23 S rRNA or 5 S rRNA are identical in all strains studied so far. Here we present data showing the presence of three regions hybridizing with 16 S rRNA and two regions hybridizing with 23 S rRNA and 5 S rRNA in M. Gallisepticum strain S6 genome.

2. MATERIALS AND METHODS

Mycoplasma Gallisepticum strain S6 was kindly provided by Prof. Freundt (FAO/WHO Collaborating Center for Animal Mycoplasmas, Arhus, Denmark). Before use the strain was purified by the three steps filtration-cloning procedure [4]. Cells were grown in modified Hayflick medium [4].

Mycoplasma DNA was isolated by Gross-Bellard method [4]. For Southern hybridization 2 μg of Mycoplasma DNA was digested with 20 units of a restriction enzyme for 1 h under conditions recommended by the manufacturer (New England BioLabs).

Southern hybridization, genomic library preparation and screening, and characterization of the clones selected were made as previously described [5]. All other molecular biology techniques were performed according to Maniatis et al. [6].

Plasmids pSB 1 and pKS 1 were made by insertion of 2 kb *Sal*I-*Bam*HI fragment (Fig. 1) or 1.5 kb *Kpn*I-*Sph*I fragment (Fig. 1) from lambda MG 7 [5] into plasmid vector pTZ 18R (Pharmacia).

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3. RESULTS

Our previous paper has described the cloning of classical rRNA operon of M. Gallisepticum on the basis of hybridization screening of M. Gallisepticum genomic library with 16 S rRNA and 23 S rRNA *E. coli* [5]. The following plasmids carrying DNA fragments (Fig. 1) from previously described phage MG 7 [5] were used in this study: (i) pSB 1, carrying 2 kb *Sal*I-*Bam*HI fragment as a probe for 23 S rRNA (the inner part of 23 S rRNA gene); (ii) pKS 1, carrying 1.5 kb *Kpn*I-*Sph*I fragment as a probe for 16 S rRNA (the coding sequence for 16 S rRNA and approximately 200 bp of 5′-noncoding sequence). Pilot experiments indicated that the hybridization patterns of digested M. Gallisepticum DNA with the probes described are identical to that obtained utilizing 23 S rRNA or 16 S rRNA *E. coli* as probes. Oligonucleotide A01 (ACACCTGGTCCCATTTCCGAACCC) synthesized according to published sequence of 5 S RNA of M. Gallisepticum [7] was used as a probe for 5 S RNA.

To estimate the number of rRNA genes, M. Gallisepticum DNA was digested with *Bgl*II, *Hin*dIII or *Eco*RV and Southern hybridization was performed under high stringency conditions [5] with the probes for 16 S rRNA (Fig. 2A, Fig. 3B, line 8), 23 S rRNA (Fig. 2B, Fig. 4A, 4B, line 8) and 5 S rRNA.

As one can see from Fig. 2B, 23 S rRNA probe reveals two hybridizing fragments in either *Bgl*II or *Eco*RV digest of Mycoplasma Gallisepticum DNA. Neither *Bgl*II nor *Eco*RV are present within the 23 S

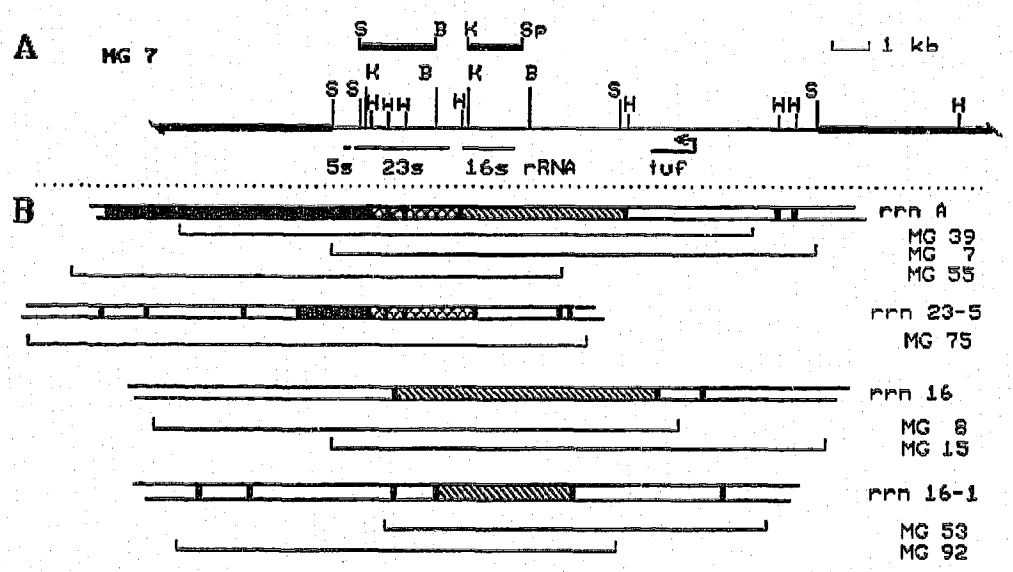

Fig. 1. M. Gallisepticum regions hybridizing with rRNA probes. (A) Restriction map of MG7 clone (middle) and fragments (top) used as a hybridization probes for 23 S rRNA (S-B) and 16 S (K-Sp) rRNA. On the bottom the position of the mapped genes [5] is shown. S=*Sal*I; B=*Bam*HI; K=*Kpn*I; Sp=*Sph*I, H=*Hin*dIII. (B) The *Hin*dIII sites localization in M. Gallisepticum genome regions hybridizing with rRNA probes. rrn A = unsplit operon (covered by group 1 clones); rrn 23-5 = region containing 23 S and 5 S hybridizing fragments (group 2); rrn 16 (group 3) and rrn 16-1 (group 4) = regions containing 16 S hybridizing fragment. The boundaries of inserts in lambda clones are shown under the maps of corresponding regions. (▨) fragment hybridizing with 16 S rRNA; (▩) fragment hybridizing with 23 S rRNA; (■) fragment hybridizing with both 23 S and 5 S rRNA.

rRNA probe. Five bands appear in hybridization of *Hin*dIII digested Mycoplasma DNA with 23 S rRNA probe (Fig. 4B, track 8). Presence of 3 *Hin*dIII sites inside the 23 S RNA coding sequence was demonstrated previously [5].

Three hybridizing fragments are revealed by 16 S rRNA probe in all digests (Figs. 2A, 3B).

Hybridization of digested DNA with 5 S rRNA probe reveals two bands in all digests. Moreover, the length of 5 S rRNA hybridizing fragments in *Bgl*II digest of M. Gallisepticum DNA is identical to that of 23 S rRNA hybridizing fragments (not shown). Two fragments revealed by 5 S rRNA probe (2 kb and 7.5 kb) in *Hin*dIII digested M. Gallisepticum DNA are identical in length with two out of five fragments revealed by 23 S rRNA probe (Fig. 4C). 5 S rRNA hybridizing *Eco*RV fragments are not identical to the 23 S rRNA hybridizing fragments (results not shown; the presence of *Eco*RV site in 5 S rRNA sequence of M. Gallisepticum was shown in [7]).

We conclude that: there are two 23 S–5 S and three 16 S hybridizing regions in M. Gallisepticum genome.

In order to confirm the interpretation, M. Gallisepticum genomic library was screened with 23 S rRNA and 16 S rRNA probes. Eight positive clones were selected and the restriction maps of the selected clones were determined. According to restriction maps, clones can be subdivided into 4 groups (Fig. 1): (i) MG 7, MG 39 and MG 55; (ii) MG 75; (iii) MG 53 and MG 92; (iv) MG 8 and MG 15. The genome fragments covered by members of each group overlap. The regions covered by members of different groups do not overlap.

The hybridization of *Hin*dIII and *Bgl*II digested DNA from clones with 16 S rRNA probe and 23 S rRNA probe is shown in Figs. 3 and 4. The members of the 1st group possess both 23 S and 16 S hybridizing fragments (described in detail in [5]). Clone MG 75 has only 23 S and 5 S hybridizing fragments; there is no fragment which hybridizes with 16 S rRNA probe. The members of the 3rd (rrn 16) and the 4th (rrn 16-1) groups contain only 16 S rRNA hybridizing fragments and no fragments hybridizing with 23 S and 5 S rRNA probes.

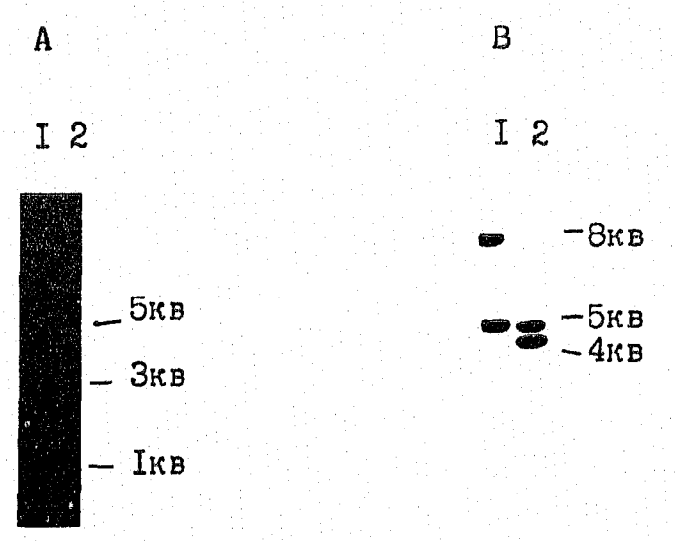

Fig. 2. Estimation of the number of 16 S rRNA and 23 S rRNA genes in DNA of M. Gallisepticum. DNA of M. Gallisepticum was digested by *Eco*RV (1) or *Bgl*II (2). Hybridization was performed with 16 S rRNA probe (A) and 23 S rRNA probe (B).

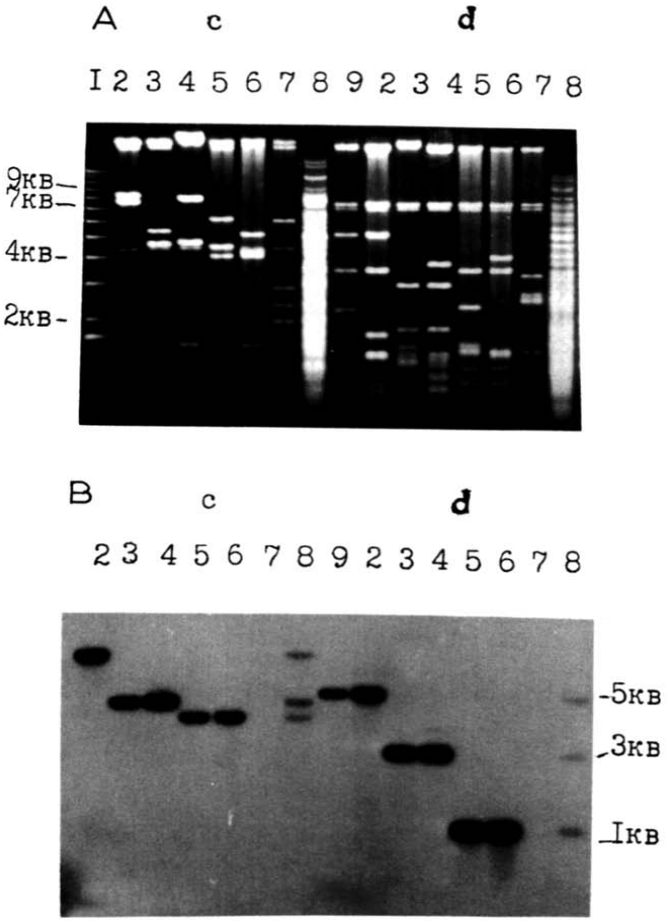

Fig. 3. Analysis of 16 S rRNA and 23 S rRNA hybridizing regions in M. Gallisepticum DNA. M. Gallisepticum DNA (8) and DNA from MG 15 (2), MG 8 (9), MG 7 (3), MG 39 (4), MG 53 (5), MG 92 (6), MG 77 (7) was digested by *Hin*dIII (c) and *Bgl*II (d), electrophoresed in 0.6% agarose gel (A). Blot hybridization was performed with 16 S rRNA probe (B). 1 = 1 kb ladder (BRL).

The pattern of hybridization is identical for all clones inside the group and is specific for each group. As one can see, each hybridizing band appearing in each digest of M. Gallisepticum DNA has the corresponding band in the cloned regions, wich not a single one of the genomic bands missing in the cloned regions (Figs. 1, 3, 4).

To summarize the above results the following conclusion can be drawn.

In addition to the unsplit operon of rRNA genes (16 S–23 S–5 S) M. Gallisepticum genome contains two separate regions hybridizing with 16 S rRNA and one region 23 S–5 S rRNA. The distances between these regions are no less than 6 kb (Fig. 1). We avoid calling the hybridizing regions genes, because additional experiments (sequencing as well as the analysis of transcription) are required to exclude the presence of pseudogenes in hybridizing regions (now in progress).

4. DISCUSSION

The attempts to estimate the number of rRNA genes in genome of M. Gallisepticum on the basis of hybridization of digested DNA with rRNA specific probes were made in several investigations [3,8]. The following interpretation of the results has been made. (i) There are two sets of 16 S rRNA, 23 S rRNA, 5 S rRNA genes in genome. (ii) One set of genes is organized in common for eubacteria rRNA operon: 16 S–23 S–5 S. The gene for 16 S rRNA in the second set is separated from either of the 23 S–5 S rRNA genes or unsplit operon for a distance of more than 80 kb.

The results presented here and in our previous paper confirm the above conclusions about the organization

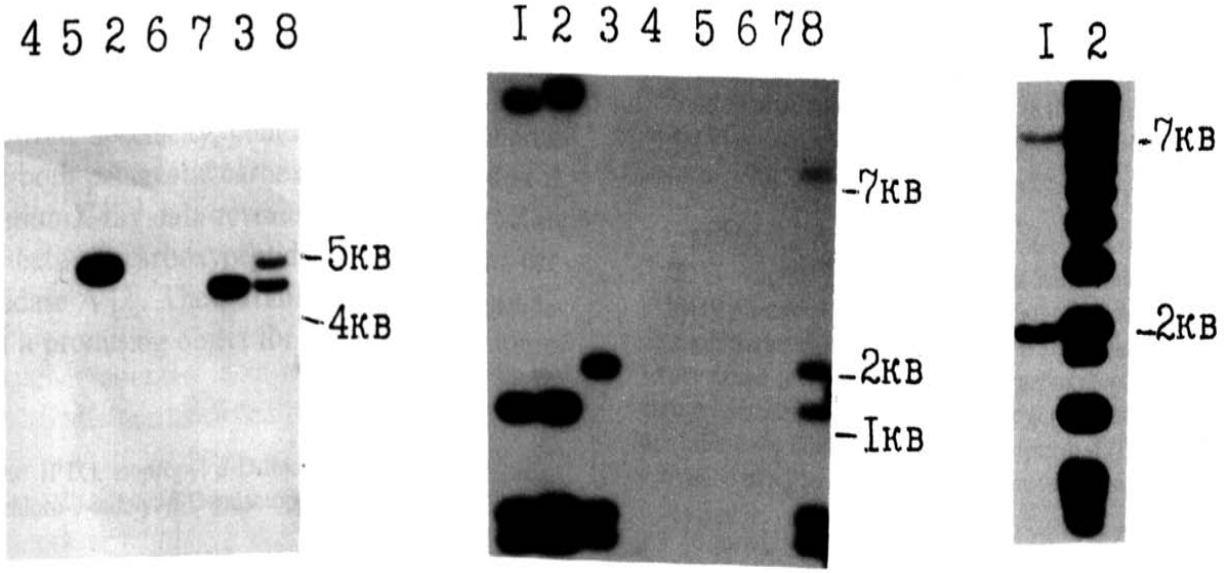

Fig. 4. Analysis of 16 S rRNA, 23 S rRNA and 5 S rRNA hybridizing regions in M. Gallisepticum DNA. (A,B) M. Gallisepticum DNA (8) and DNA from MG 7 (1), MG 39 (2), MG 77 (3), MG 8 (4), MG 15 (5), MG 53 (6), MG 92 (7) was digested by *Hin*dIII (B), *Bgl*II (A) and blot-hybridized with 23 S rRNA probe. (C) M. Gallisepticum DNA (1) was digested by *Hin*dIII and blot-hybridized with 5 S rRNA probe. 2 = 1 kb ladder (BRL).

of putative rRNA genes in operons, as well as conclusions about the number of 23 S rRNA and 5 S rRNA hybridizing regions, but lead us to conclude about the existence of the third region hybridizing with the 16 S rRNA probe. Nevertheless as far as the results of experiments reported by Amikan et al. are concerned, one can see that according to their Table I and Fig. 2 [8], the hybridization of *Eco*RI digested M. Gallisepticum strain A5969 DNA with 16 S rRNA probe produces 3 bands. Our interpretation (existence of 3rd 16 S rRNA hybridizing regions in M. Gallisepticum genome) seems to be more natural than that suggested by authors (existence of *Eco*RI site in one among two 16 S rRNA genes). Moreover, we have not found *Eco*RI sites inside the cloned 16 S rRNA hybridizing regions. However, it is worth noting that our variant of S6 strain seems to be more similar to the strain A5969 than to S6 variant studied by Amikan et al. according to their hybridization response.

To explain the results reported by Chen et al. [3] it is necessary to assume that the unsplit rRNA operon of M. Gallisepticum should be situated at a distance less than 50 kb from the isolated 16 S rRNA hybridizing region with no sites for *Bgl*I and *Bss*HII between them.

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