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# A Physical Map of the Sulfur-Dependent Archaebacterium *Sulfolobus acidocaldarius* 7 Chromosome

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**A chromosomal map of the sulfur-dependent thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* 7 was constructed with four restriction enzymes: *Not*I, *Bss*HII, *Rsr*II, and *Eag*I. The map indicated that the chromosome is a single circular DNA of 2,760 ± 20 kb (mean ± standard error of the mean). rRNA genes were also mapped. They were located at one site in the genome.**

Pulsed-field gel electrophoresis (PFGE) has been used to analyze eubacterial chromosomal DNAs. Restriction maps of several eubacterial chromosomes have been reported (2, 4, 6, 8, 13–15, 20–22, 24, 25) and were reviewed recently (16). Maps are circular in almost all cases.

K. M. Noll reported the circular chromosome map of the thermophilic archaebacterium *Thermococcus celer* (19). Complete physical maps were also reported for *Methanococcus voltae* (23), *Haloferax volcanii* DS2 (7), and *Haloferax mediterranei* (17). We analyzed the chromosomal DNA of the sulfur-dependent thermoacidophilic archaebacterium *Sulfolobus acidocaldarius* 7 with the restriction enzyme *Not*I and *Not*I-linking clones and clearly proved that the chromosomal DNA is circular (29). We present a more precise restriction map in this report. The map showed that the chromosome is a single circular DNA of 2,760 ± 20 kb (mean ± standard error of the mean). We also mapped the *rrn* genes.

**Preparation of intact chromosomal DNA.** *S. acidocaldarius* 7 (26) was cultured in the basal salt medium of Brock et al. (3) supplemented with 0.1% tryptone. DNA samples were prepared in agarose blocks as described previously (29).

**Digestion of the DNA in agarose blocks and electrophoresis.** Agarose blocks were washed with TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA) for 3 h on ice with six buffer exchanges and then equilibrated with suitable enzyme buffer for 1 h with a buffer exchange. Each agarose block was treated with 10 to 20 U of restriction enzyme at the appropriate temperature for 2 to 4 h.

PFGE was performed on a 1.2% agarose gel in 0.5× TEB buffer (44.5 mM Tris base, 44.5 mM boric acid, 1 mM EDTA) at 5.7 V/cm by using a hexagonal system (30) at 16°C for an appropriate period, depending on the fragment size. Lambda DNA ladders and the chromosomal DNA of *Saccharomyces cerevisiae* S288C were prepared as described in references 1 and 5, respectively.

**Preparation of rRNA.** Ribosomes were prepared in solution A, which contained 20 mM Tris-HCl, pH 7.6, 10 mM Mg acetate, and 20 mM $NH_4Cl$. Cells were suspended in solution A and lysed by passage through a French pressure cell. After the addition of DNase, the cell lysate was cleared by centrifugation at 30,000 × *g* for 30 min. Ribosomes were recovered from the cleared lysate and washed once with solution A by centrifugation at 180,000 × *g* for 3 h. The precipitate was suspended in solution B, which contained 10 mM Tris-HCl, pH 7.6, and 0.2 mM Mg acetate. Subunits were separated in a 10 to 30% sucrose density gradient in buffer B by centrifugation in a Beckman SW28 rotor for 16 h at 21,000 rpm. RNA was purified by phenol extraction and recovered by ethanol precipitation. The rRNA was further purified by gel electrophoresis.

**Southern blot analysis.** Southern blot analysis was done according to the standard procedure (18) as described previously (29). A Fujix Bio-imaging Analyzer was used to detect weaker isotope signals. For isotopic labeling of probes, DNA bands were cut out after electrophoresis and DNA was extracted by using phenol from low-melting-temperature agarose gel (Bio-Rad) or by using glass milk (Bio 101, Inc.) from normal agarose gel.

Alternatively, the low-melting-temperature agarose blocks containing DNA bands were labeled directly. They were labeled by using a random primer labeling kit (Takara Shuzou). When agarose blocks were labeled directly, they were melted and 12 μl was mixed with 2 μl of primer solution and annealed. Agarose was solidified again in the annealing procedure, and reaction buffer, deoxynucleoside triphosphate, [$^{32}$P]dCTP, and the Klenow fragment of DNA polymerase I were added. The mixture was incubated at 37°C for 5 h. After the addition of 225 μl of TE buffer, gels were melted at 65°C and applied to a small gel filtration column (Sephadex G50).

For preparation of 16S and 23S rRNA probes, RAV-2 reverse transcriptase (Takara Shuzou) was used instead of the Klenow fragment. The labeled mixtures were heat denatured at 100°C for 3 min, treated with RNase A, and extracted with phenol before gel filtration. 5S rRNA was dephosphorylated and labeled with polynucleotide kinase at its 5′ end.

**Restriction enzymes.** About 20 restriction enzymes were tested considering the relatively low G+C content, 40% (29), of the organism. Among those tested, eight enzymes (*Apa*I, *Fsp*I, *Nae*I, *Nar*I, *Nru*I, *Sma*I, *Mlu*I, and *Xho*I) yielded fragments greater than 200 kb in size, although there were more than 20 fragments. Four restriction enzymes (*Bss*HII, *Eag*I, *Not*I, and *Rsr*II) yielded fewer than about 20 fragments and were used for construction of the physical map.

**Estimation of the size of the *S. acidocaldarius* 7 chromosome.** We have previously reported that the 8-base-recognition enzyme *Not*I cut the *S. acidocaldarius* 7 chromosomal DNA into two bands and that the sum of the fragment lengths was about 3,100 kb (29). The restriction enzymes *Bss*HII, *Eag*I, and *Rsr*II yielded 6, 20, and 12 bands, respectively, with the fragment size ranging from 6 to 945 kb

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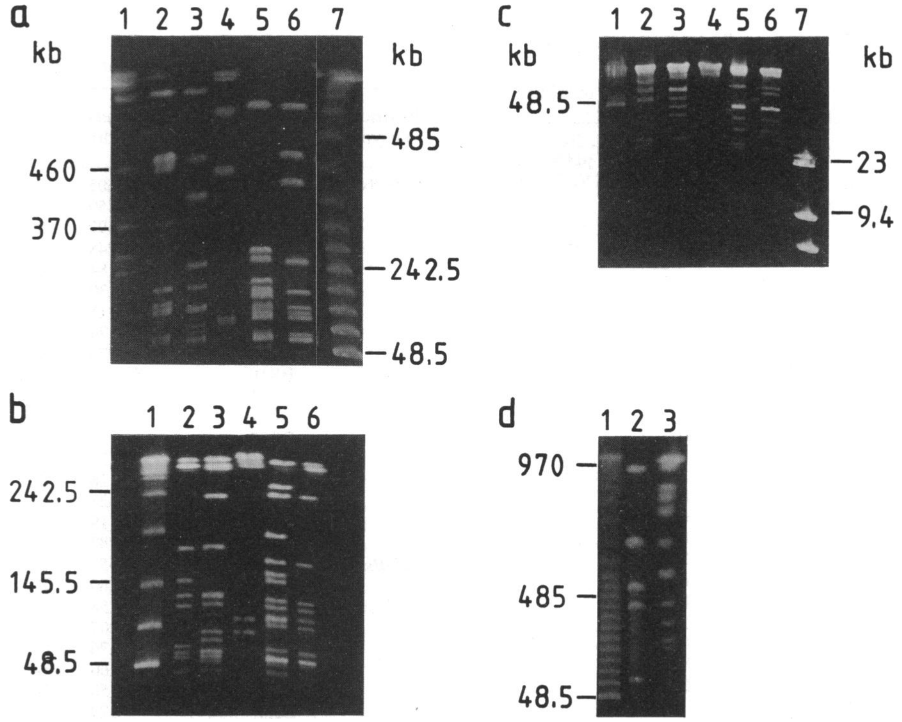

FIG. 1. Separation of DNA fragments of the *S. acidocaldarius* 7 chromosome by PFGE. (a) 60-s pulses, 35 h; (b) 20-s pulses, 24 h; (c) 3-s pulses, 14 h; (d) 100-s pulses, 35 h. Lanes: 2 (except in panel d), digestion of *S. acidocaldarius* DNA with *Rsr*II; 3 (except in panel d), digestion with *Rsr*II-*Bss*HII; 4 (and lane 2 in panel d), digestion with *Bss*HII; 5, digestion with *Bss*HII-*Eag*I; 6, digestion with *Eag*I. Size markers: lane 1 in panel a and lane 3 in panel d, *S. cerevisiae* DNA; lane 1 in panels b to d and lane 7 in panel a, lambda ladders; lane 7 in panel c, *Hin*dIII digest of lambda DNA.

(Fig. 1 and Table 1). The size was estimated from the electrophoretic separation pattern with an appropriate pulse time for each fragment size. A lambda ladder size marker was used to estimate the sizes of all the fragments except *Not*I. The total lengths of the fragments were 2,812, 2,780, and 2,708 kb from *Bss*HII, *Rsr*II, and *Eag*I digestion, respectively. The sizes of fragments resulting from *Bss*HII-*Rsr*II and *Bss*HII-*Eag*I double digestion are summarized in Table 2. The total lengths of these fragments were 2,782 and 2,728 kb, respectively. By averaging these five values, we estimated the chromosomal size to be 2,760 ± 20 kb (mean ± standard error of the mean).

**Physical map of the *S. acidocaldarius* 7 chromosome.** These gels were transferred to a membrane and analyzed by using several DNA fragments as probes. Results of these Southern blot analyses are summarized in Tables 1 and 2. Six *Bss*HII fragments were primarily used as probes. *Not*I-linking clones (29) and *Rsr*II bands were also used. Each of the four *Not*I-linking clones, pHNS7, pHNS7R, pXNC7, and pXNL7, harbors each arm of the two *Not*I-linking fragments, pHN7 and pXN7 (29).

Every fragment of the *Rsr*II and *Eag*I digests was detected by at least one of these probes. From these hybridization data and the estimated sizes of fragments, each fragment after double digestion was assigned to the original fragment resulting from single digestion (Table 2). These results were used for restriction map construction. Every fragment was uniquely aligned in a circular form (Fig. 2). Because *Eag*I recognizes the inner six bases d(CGGCCG) of the *Not*I recognition sequence d(GCGGCCGC), *Not*I-linking clones could be used to align four *Eag*I fragments. Although the order of a few fragments, i.e., R9/R10, R11/R12, E10/E19, E12b/E16/E18, and E14/E17, are still unsure, the position of each pair or triplet was uniquely determined.

**Physical mapping of rRNA genes.** Figure 3 shows the

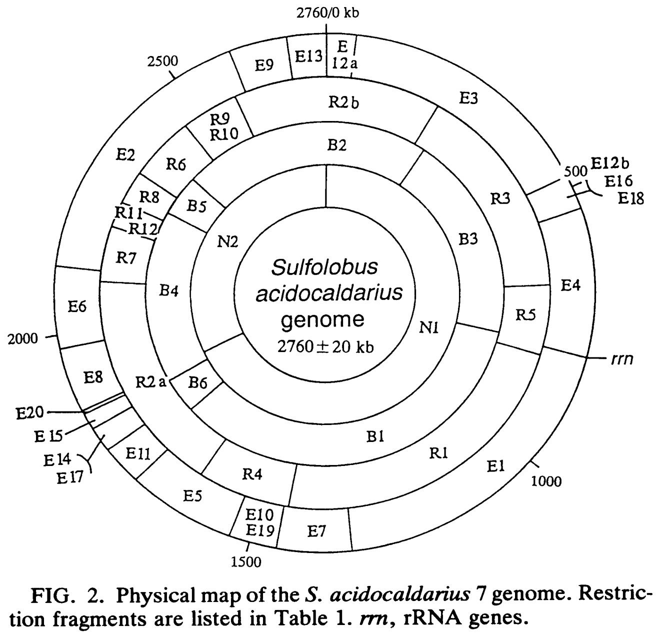

FIG. 2. Physical map of the *S. acidocaldarius* 7 genome. Restriction fragments are listed in Table 1. *rrn*, rRNA genes.

TABLE 1. Sizes of and hybridization data for the restriction fragments of the *S. acidocaldarius* 7 chromosomal DNA

| Restriction enzyme | Fragment(s) | Size (kb)[a] | Probe(s) hybridized[b] |
|---|---|---|---|
| *Not*I | N1 | 2,100 | B1, B2, B3, B4, pXNC7, pHNS7R |
| | N2 | 1,000 | B2, B4, pXNL7, pHNS7 |
| | Total size | 3,100 | |
| *Bss*HII | B1 | 945 | R1, R4, R5, 5S, 16S, 23S |
| | B2 | 693 | |
| | B3 | 540 | R5, 23S |
| | B4 | 442 | R7, pHNS7, pHNS7R |
| | B5 | 105 | |
| | B6 | 87 | |
| | Total size | 2,812 | |
| *Rsr*II | R1 | 605 | B1 |
| | R2a, R2b | 459, 459 | B1, B2, B4, B6, pHNS7, pHNS7R |
| | R3 | 445 | B2, B3 |
| | R4 | 184 | B1 |
| | R5 | 146 | B1, B3, 5S, 16S, 23S |
| | R6 | 130 | B2, B5 |
| | R7 | 115 | B4 |
| | R8 | 69 | B4, B5 |
| | R9 | 60 | B2 |
| | R10 | 55 | B2 |
| | R11 | 34 | B4 |
| | R12 | 19 | B4 |
| | Total size | 2,780 | |
| *Eag*I | E1 | 557 | B1, R1, 16S, 5S |
| | E2 | 460 | B2, B4, B5, R7 |
| | E3 | 421 | B2, B3 |
| | E4 | 258 | B3, R5, 23S |
| | E5 | 176 | B1, R4 |
| | E6 | 129 | B4, R7 |
| | E7 | 121 | B1, R1, R4 |
| | E8 | 105 | B4, pHNS7 |
| | E9 | 93 | B2 |
| | E10 | 69 | B1, R4 |
| | E11 | 66 | B1, B6 |
| | E12a, E12b | 47,47 | B2, B3, pXNC7 |
| | E13 | 45 | B2, pXNL7 |
| | E14 | 40 | B6 |
| | E15 | 32 | B4, B6 |
| | E16 | 11 | B3 |
| | E17 | 9 | B6 |
| | E18 | 8 | B3 |
| | E19 | 7 | R4 |
| | E20 | 6 | pHNS7R |
| | E21 | 1.4 | 23S |
| | Total | 2,708 | |

[a] Sizes of the *Not*I fragments were determined by using *S. cerevisiae* and *Schizosaccharomyces pombe* size markers described in reference 29 and may not be very accurate.

[b] Probes used were *Bss*HII and *Rsr*II fragments of *S. acidocaldarius* 7 DNA and *Not*I-linking clones and rRNAs. Each of the *Not*I-linking clones, pXNC7, pXNL7, pHNS7 and pHNS7R, harbors each arm of the two *Not*I-linking fragments, pXN7 and pHN7 (29).

TABLE 2. Fragment sizes and the results of hybridization analysis of the fragments after double digestion of the *S. acidocaldarius* 7 DNA

| Restriction enzymes | Fragment(s) | Size(s) (kb) | Probe(s) hybridized[a] | Original fragment(s) assigned[b] |
|---|---|---|---|---|
| *Rsr*II-*Bss*HII | BR1 | 605 | B1 | R1 |
| | BR2 | 459 | B2 | R2b |
| | BR3 | 397 | B3 | R3 |
| | BR4 | 248 | B4 | R2a |
| | BR5 | 184 | B1 | R4 |
| | BR6 | 130,130 | B1, B3, R5, 23S | R5, R2a |
| | BR7 | 115 | B4 | R7 |
| | BR8 | 76 | B6 | R2a |
| | BR9 | 70 | B2 | R6 |
| | BR10 | 60, 60 | B2, B5 | R6, R9 |
| | BR11 | 55 | B2 | R10 |
| | BR12 | 48 | B2 | R3 |
| | BR13 | 43 | B5 | R8 |
| | BR14 | 34 | B4 | R11 |
| | BR15 | 26 | B4 | R8 |
| | BR16 | 23 | R5, 5S, 16S, 23S | R5 |
| | BR17 | 19 | B4 | R12 |
| | Total size | 2,782 | | |
| *Eag*I-*Bss*HII | BE1 | 557 | B1, R1, 5S, 16S | E1 |
| | BE2 | 280 | B3 | E3 |
| | BE3 | 258 | B3, 23S | E4 |
| | BE4 | 207 | B2 | E2 |
| | BE5 | 176 | B1, R4 | E5 |
| | BE6 | 160 | B4, R7 | E2 |
| | BE7 | 150 | B2 | E3 |
| | BE8 | 129 | B4, R7 | E6 |
| | BE9 | 121 | B1, R1, R4 | E7 |
| | BE10a, BE10b | 105, 105 | B4, B5, pHNS7 | E8, E2 |
| | BE11 | 93 | B2 | E9 |
| | BE12 | 69 | B1, R4 | E10 |
| | BE13a, BE13b | 47, 47 | B2, B3, pXNC7 | E12a, E12b |
| | BE14 | 45 | B2, pXNL7 | E13 |
| | BE15 | 40 | B6 | E14 |
| | BE16 | 34 | B6 | E11 |
| | BE17 | 32 | B1 | E11 |
| | BE18 | 21 | B4 | E15 |
| | BE19 | 11 | B3 | E16 |
| | BE20 | 10 | B6 | E15 |
| | BE21 | 9 | B6 | E17 |
| | BE22 | 8 | B3 | E18 |
| | BE23 | 7 | R4 | E19 |
| | BE24 | 6 | pHNS7R | E20 |
| | BE25 | 1.4 | 23S | E21 |
| | Total size | 2,728 | | |

[a] Probes were the same as those in Table 1.

[b] Original *Rsr*II or *Eag*I fragments were assigned on the basis of the size and the hybridization data.

Southern blot analysis of the fragment after *Eco*RI, *Bam*HI, *Hin*dIII, and *Xba*I digestion. Only one band was detected by rRNA probes in each lane. Accordingly, these rRNA genes are located within these fragments, i.e., 8.0-kb *Eco*RI, 11.5-kb *Bam*HI, 9.0-kb *Hin*dIII, and 7.0-kb *Xba*I fragments. Additional bands were detected by using a 5S rRNA probe after *Bam*HI digestion and *Xba*I digestion: a 1.0-kb *Bam*HI band and a 4.3-kb *Xba*I band, respectively.

Figure 4 shows that rRNA genes are located at or near the interface between E4 and E1 or between B1 and B3 within the R5 fragment. An additional band, designated E21, was also detected by 23S rRNA. The relative position of the band changed in different electrophoresis runs. The band position shown in Fig. 4A corresponds to a position between E10 and E12. The apparent size estimated here, 50 to 60 kb, could not be reconciled with the fact that 23S rRNA hybridized with only one band having a size ranging from 7 to 11 kb in Fig. 3. We believe that the band labeled E21 corresponds to the band that migrated at 1.4 kb after normal gel electrophoresis, as shown in Fig. 5.

After *Taq*I digestion, one strong 1.8-kb band and three weaker bands (1.3, 1.0, and 0.3 kb) were detected by 23S rRNA (Fig. 5). Signals were detected at 1.8 and 0.2 kb by 16S rRNA and at 1.8 and 0.4 kb by 5S rRNA. The 1.8-kb fragment that hybridized with 23S rRNA was different from the band detected by 16S and 5S rRNA, because the former fragment was cleaved by *Eag*I, in contrast to the latter. None of these bands changed its mobility by the additional treatment with *Xba*I. These hybridization experiments

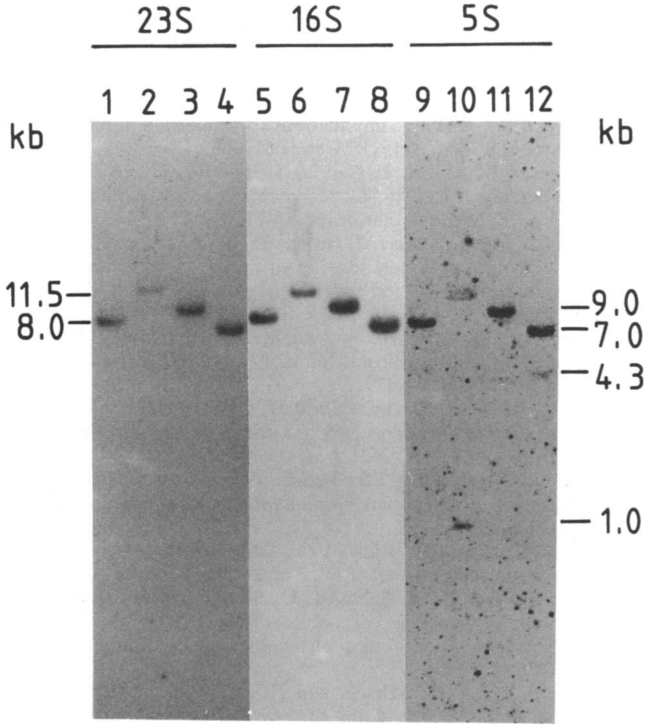

FIG. 3. Southern blot analysis of rRNA genes. *Eco*RI (lanes 1, 5, and 9), *Bam*HI (lanes 2, 6, and 10), *Hin*dIII (lanes 3, 7, and 11), and *Xba*I (lanes 4, 8, and 12) digests of *S. acidocaldarius* 7 DNA were electrophoresed in a 1.2% agarose gel and analyzed with rRNA probes shown above the lane numbers.

showed that the genes are adjacent to one another and are a single copy. The position is indicated in Fig. 2. The relative order of rRNA genes will be analyzed precisely elsewhere.

**Circular chromosome in the last common ancestor.** The recent molecular evolutional analysis of H$^+$-ATPases (10) and other paralogous genes (12, 27) revealed the position of the archaebacterial group in the evolutionary tree. The archaebacterial group is likely to be situated in the same branch as eukaryotes. The archaebacterial group is divided into two subgroups; one is of sulfur-dependent thermophiles, including *S. acidocaldarius*, and is named Crenarchaeota, and another is of methanogens, including halophiles and *T. celer*, and is named Euryarchaeota (27, 28).

Noll has reported the circular chromosomal map of *T. celer* (19). Circularity of the chromosome was also shown for *M. voltae* (23), *H. mediterranei* (17), and *H. volcanii* DS2 (7). These organisms belong to Euryarchaeota. We have clearly proved the circularity of the *S. acidocaldarius* 7 chromosome (29); the organism belongs to Crenarchaeota. The complete physical map obtained here supported the circularity of the chromosome. These findings suggest the general occurrence of circular chromosomes in archaebacterial species.

Increasing numbers of reports of eubacterial physical maps indicate the general occurrence of circular chromosomes (2, 4, 6, 8, 13–15, 20–22, 24, 25; for a review, see reference 16). Although there is the report of linear DNA in *Borrelia burgdorferi*, it is perhaps unique among eubacteria (9). The common occurrence of circular chromosomes in both eubacteria and archaebacteria supports our previous proposal that the last, or the most recent, common ancestor of all the living organisms on the earth had a circular chromosome (29). Thus, at the time when eubacteria and archaebacteria diverged, life on the earth had already developed the rigid DNA-based genetic system with circular chromosomal DNA.

*S. acidocaldarius*, *M. voltae*, and *T. celer* contain one chromosome. However, halophiles have a more complex chromosome structure, having plasmids larger than 100 kb (7, 11, 17). A photosynthetic eubacterium, *Rhodobacter sphaeroides*, is reported to have two unique circular chro-

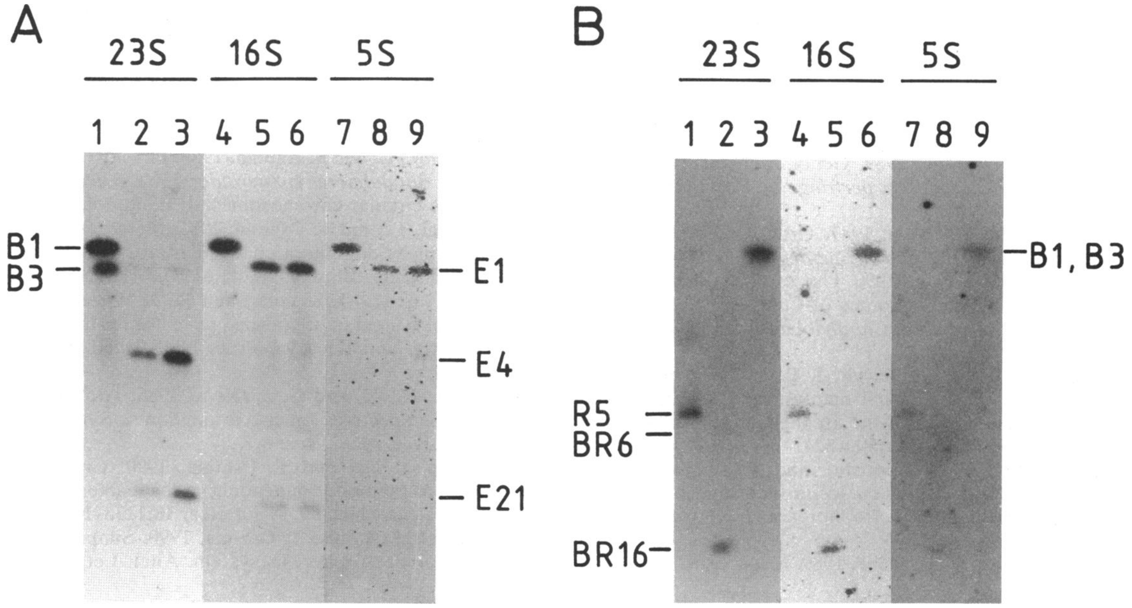

FIG. 4. Southern blot analysis of rRNA genes after PFGE. (A) *S. acidocaldarius* 7 DNA was digested with *Bss*HII (lanes 1, 4, and 7), *Bss*HII plus *Eag*I (lanes 2, 5, and 8), or *Eag*I (lanes 3, 6, and 9) with 60-s pulses for 21 h and 10-s pulses for 22 h. (B) The Enzyme used was *Rsr*II (lanes 1, 4, and 7), *Rsr*II plus *Bss*HII (lanes 2, 5, and 8), or *Bss*HII (lanes 3, 6, and 9); 20-s pulses, 40 h.

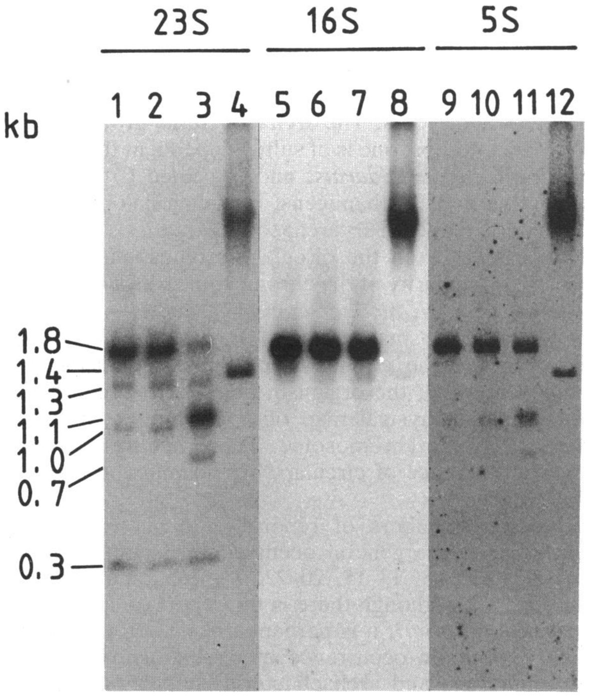

FIG. 5. Southern blot analysis of a *Taq*I digest of *S. acidocaldarius* 7 DNA. Enzymes used were *Taq*I (lanes 2, 6, and 10), *Taq*I and *Xba*I (lanes 1, 5, and 9), *Taq*I and *Eag*I (lanes 3, 7, and 11), and *Eag*I (lanes 4, 8, and 12). Normal 1.5% agarose gel electrophoresis.

mosomes (25). Accordingly, the number of chromosomes may not be meaningful in both archaebacteria and eubacteria.

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