Genomic organization of rDNA in *Pseudomonas aeruginosa*

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We have examined the number and organization of rRNA genes in *Pseudomonas aeruginosa* by hybridization of restriction nuclease digests of genomic DNA to 3'-^{32}P-labelled 23 S, 16 S and 5 S rRNAs and corresponding labelled DNA from the rrnB operon of *Escherichia coli*. The immediate conclusion from these hybridization data is that there are 4 transcriptional units coding for rDNA in *P. aeruginosa*. We report here a putative model of the genomic organization of all 4 rDNA operons.

(Pseudomonas aeruginosa) *rDNA* *rRNA gene* *DNA-DNA hybridization* *RNA-DNA hybridization*

1. INTRODUCTION

All known eubacterial rDNAs are organized as operons with the typical gene sequence 5'-16 S-23 S-5 S-3'. Studied examples of multiple gene copies, in *Escherichia coli*, include in particular 7 rRNA operons rrnA to rrnG. In each transcriptional unit, there is one gene for each rRNA, with the exception of rrnD, where two 5 S rRNA genes are organized tandemly at the end of the operon [1]. Although located at 7 different map locations, the corresponding genes are nearly homologous but in general not identical [2–6]. Another remarkable aspect of some of these operons is that the rRNA genes are coupled to tRNA genes, which differ in number and species from operon to operon [5,7]. Thus, not only have the rRNA genes undergone what appears to be gene duplication in the course of evolution, but also aspects of the organization of these genes appear to have been preserved. Among eubacterial species the number of rRNA operons varies, i.e. *Mycoplasma myocides* ssp. capri [8] and *Acholeplasma laidlawii* reveal 2 operons, in *Bacillus subtilis* [9,10] each 5 S rRNA gene is closely linked to the 23 S rRNA genes and none of the 10 rRNA operons has tandemly repeated 5 S rRNA genes, whereas one operon is present in *M. capricolum* [11]. In archaebacteria: *Thermoplasma acidophilum* [12] exhibits the structural genes for the rRNAs, one per genome, in the order 5'-23 S-5 S-16 S-3', whereas in *Halobacterium halobium* [13] the rRNA cistrons are arranged in a eubacterial fashion 5'-16 S-23 S-5 S-3', having one rRNA operon per genome. Recently, a gene arrangement, having 2 operons per genome, was proposed for the eubacterium *Thermus thermophilus* [14].

2. MATERIALS AND METHODS

Media and growth conditions for *Pseudomonas aeruginosa* (ATCC 10145) were as described [15], except that cells were grown at 30°C. The rRNAs were prepared by the phenol method from 70 S ribosomes or from 50 S and 30 S ribosomal subunits [16]. The purification, polyacrylamide gel electrophoresis, 3'-end labelling and DNA preparation methods have been described in great detail [14–18] and are not reiterated. The 3'-end ^{32}P-labelled rRNAs were eluted from the gels in 10 × SSC (1 × SSC: 0.15 M NaCl, 0.015 M Na citrate). The digestions of the DNA with restriction endonucleases (see figure legends) were carried out as recommended by the manufacturers. The DNA digests were resolved by 0.7% agarose gel elec-

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trophoresis [19], subjected to denaturation by alkali treatment and transferred to nitrocellulose filters according to the Southern procedure [20]. The labelled rRNA was brought to a final radioactivity of 3×10^5 Cerenkov cpm per ml (50% formamide, 5 × SSC) and the filters were wetted with the labelled rRNA probe (approx. 15 μl per cm^2). Hybridization was carried out at 37°C for at least 18 h. After the incubation, the filter was washed twice with 5 × SSC/50% formamide for 20 min, twice with 2 × SSC for 10 min and dried for 2 h at 80°C. Radioactive bands were detected by autoradiography at −80°C with an intensifying screen and Kodak X-Omat XRP-1 film. Because of concern about cross-contamination between 16 S and 23 S rRNA we employed the method of DNA-

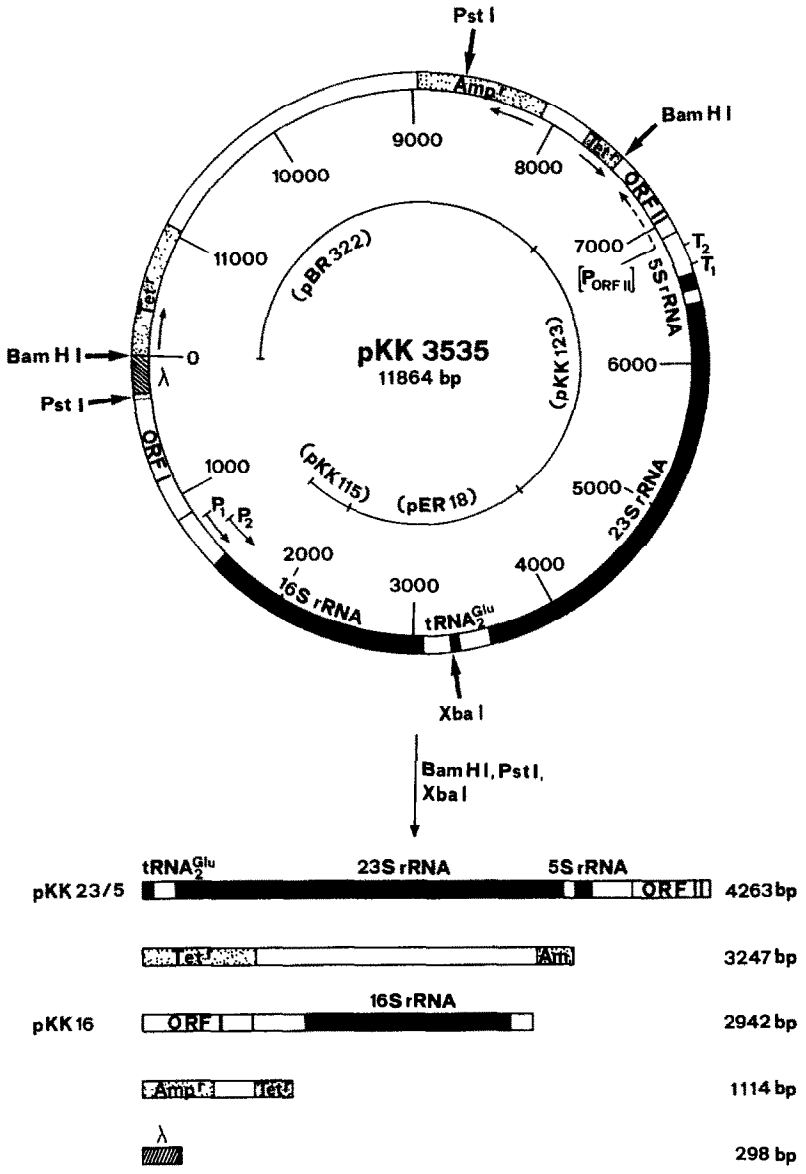

Fig. 1. Schematic representation of the plasmid pKK3535 [26], carrying the entire rrnB operon of *E. coli*. The restriction endonucleases employed to isolate rDNA and the resulting products are indicated.

DNA hybridization to verify our RNA-DNA hybridization data.

For this purpose the complete 16 S as well as the 23 S/5 S rDNA coding portion were cut out of the plasmid pKK3535 [27], a pBR322 derivative, containing the complete rrnB operon of *E. coli* (fig.1). Approx. 30 μg pKK3535 were digested with *Bam*HI, *Pst*I and *Xba*I. The fragments of interest comprising the 16 S rDNA (2942 bp) and the 23 S/5 S rDNA (4263 bp) were purified by electroelution from 0.7% agarose gels. The fragments were labelled by nick translation with [α-^{32}P]-dATP, essentially as described by Maniatis et al. [19]. Nitrocellulose filters were prehybridized overnight at 42°C with 50–100 μl/cm^2 of the following solution: 50% formamide, 5 × SSC, 5 × Denhardt, 50 mM sodium phosphate (pH 6.5), 0.1% SDS and salmon sperm DNA at 500 μg/ml.

Hybridization was carried out at 42°C for at least 18 h in a solution (25 μl/cm^2) containing 50% formamide, 5 × SSC, 1 × Denhardt, 20 mM sodium phosphate (pH 6.5), 0.1% SDS, salmon sperm DNA (100 μg/ml) and nick-translated ^{32}P-labelled DNA (3–10 × 10^5 Cerenkov cpm/120 cm^2). After the incubation the filters were washed for 15 min with 2 × SSC, 0.1% SDS at room temperature, 40 min with the same solution at 42°C and finally with 0.1 × SSC, 0.1% SDS at 42°C for 40 min.

3. RESULTS AND DISCUSSION

The pseudomonads are a group of bacteria that exhibit a wealth of exotic metabolic activities [21]. They resemble the Enterobacteriaceae morphologically, and most grow well on differential enteric media. The study of the genetics of pseudomonads began much later than that of the Enterobacteriaceae. No similarities between the linkage maps of *E. coli* and of nonenteric bacteria such as pseudomonads have been observed. Hybrid formation with rRNA demonstrated that complementary sites exist on the DNA [22]. Among bacteria belonging to the family Enterobacteriaceae, hybrid formation of rRNA with DNA of any species of the same family is almost as great as with *E. coli* DNA, and up to 50% of hybrid formation occurs with distantly related genera such as *Pseudomonas* or *Bacillus* [23]. These studies in enteric bacteria as well as those in other genera, indicate that the structure of rRNA is conserved during evolution. We looked at *P. aeruginosa* to elucidate the number of rRNA operons and tried to find evidence for the gene arrangement employing hybridization techniques.

Employing 16 S rRNA isolated from 30 S ribosomal subunits as a hybridization probe, it is of utmost importance to quench with several molar excess cold 23 S rRNA and we used in addition crude tRNA. Although we separated the 70 S ribosomes into their subunits by standard methods and pooled carefully, a 1–2% contamination with

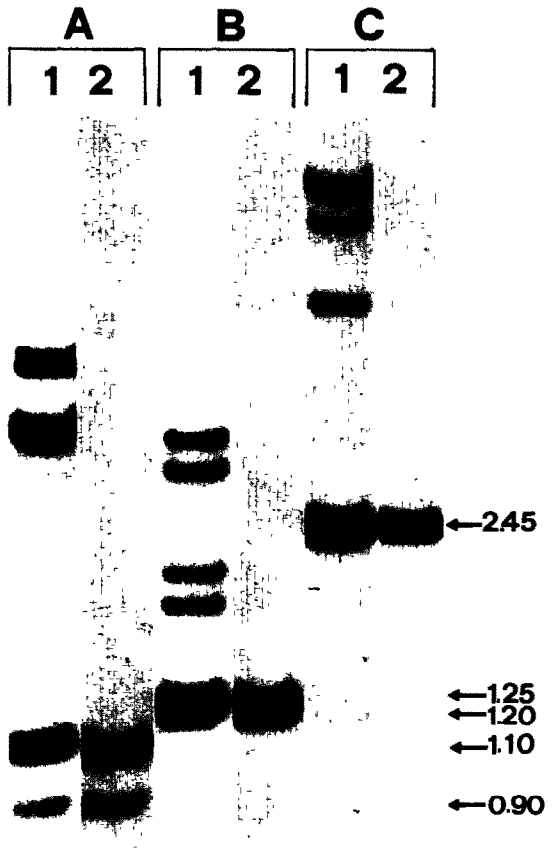

Fig.2. Autoradiographs of *P. aeruginosa* DNA fragments containing 16 S rRNA genes. The endonuclease-digested DNA was hybridized to 3′-labelled 16 S rRNA. Lanes: A, *Bam*HI-*Eco*RI; B, *Bam*HI-*Hin*dIII; C, *Hin*dIII. Lanes indicated with the number 2 represent quenched hybridization experiments, containing additionally 10 μg rRNA from *P. aeruginosa* 50 S ribosomal subunits and 40 μg tRNA from *E. coli* per μg labelled 16 S rRNA.

23 S rRNA could not be avoided, i.e. fragments which were generated from 23 S rRNA comigrated in the polyacrylamide gel electrophoresis in the range of 16 S rRNA. Fig.2 demonstrates a comparison of 16 S rRNA hybridization quenched and unquenched with unlabelled 23 S rRNA/tRNA in the hybridization probe. Because of this not yet completely resolved uncertainty we have chosen to verify our RNA hybridization data by employing in addition DNA-DNA hybridization using the plasmid pKK3535, carrying the complete rrnB transcriptional unit of *E. coli*. Knowledge of the entire restriction map allowed us to isolate DNA fragments which comprise the total 16 S rDNA and 23 S/5 S rDNA, thus avoiding as in the case of RNA hybridization any misinterpretation of hybridization data. We are certain that no contamination has affected the 5 S rRNA hybridization data (fig.3) because partial sequencing of the 5 S rRNA (not shown) before using it as a probe established its purity.

From the inspection of the 5 S rRNA sequence [24] of *P. aeruginosa* it was determined that the gene for this rRNA should not have a cleavage site which would be recognized by the restriction endonucleases (see figure legends) employed. Therefore, we concluded that a DNA fragment

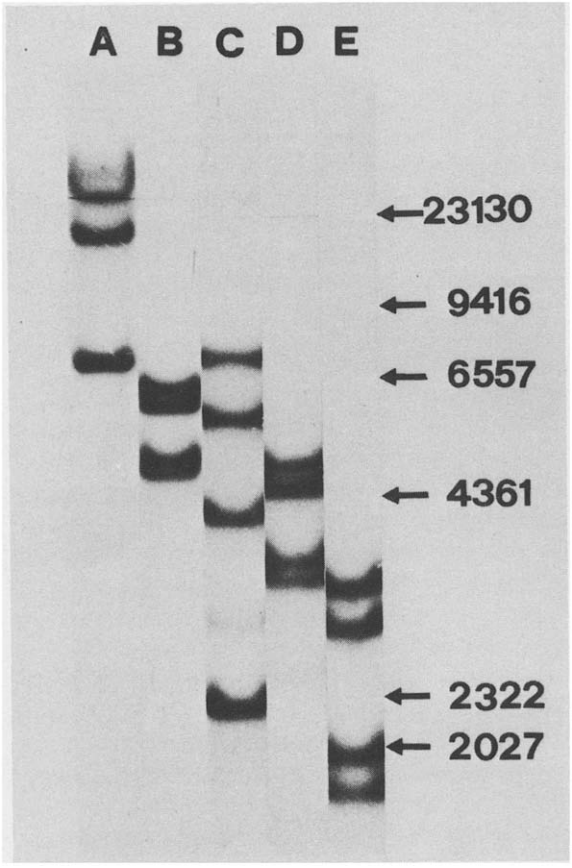

Fig.3. Autoradiographs of *P. aeruginosa* DNA fragments containing 5 S rRNA genes. The endonuclease-digested DNA was hybridized to 3′-labelled 5 S rRNA. The molecular size of the reference *Hin*dIII-lambda DNA is expressed in basepairs on the right of the column. Single digestion: (A) *Hin*dIII, (B) *Bam*HI; double digestion: (C) *Hin*dIII-*Eco*RI, (D)*Bam*HI-*Eco*RI, (E) *Bam*HI-*Hin*dIII.

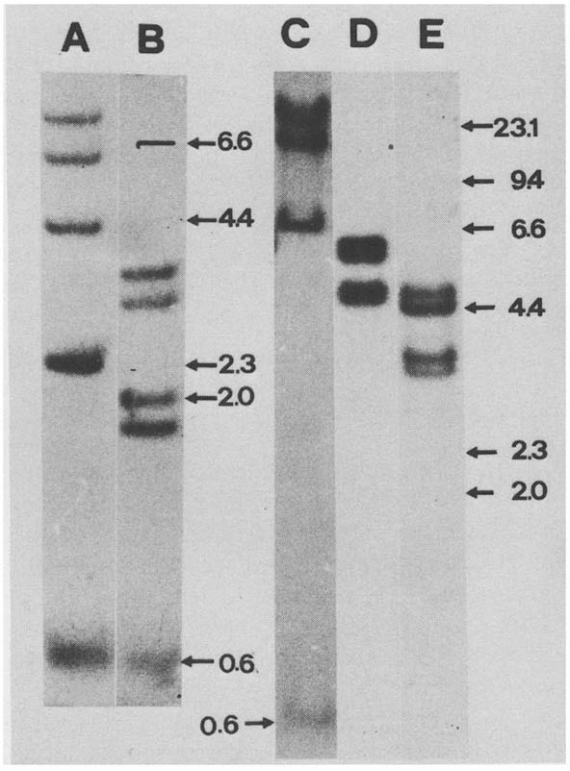

Fig.4. Autoradiographs of *P. aeruginosa* DNA fragments containing 23 S rRNA genes. The endonuclease-digested DNA was hybridized to nick-translated 23 S rDNA (pKK23/5). The molecular size of the reference is shown on the right side of the columns. Single digestion: (C) *Hin*dIII, (D) *Bam*HI; double digestion: (A) *Hin*dIII-*Eco*RI, (B) *Bam*HI-*Hin*dIII, (E) *Bam*HI-*Eco*RI.

generated by those endonucleases which will hybridize to 3′-terminus labelled 5 S rRNA must necessarily represent the whole structural gene for the 5 S rRNA.

To see whether each of these 5 S rRNA genes is linked to a 23 S rRNA gene required us to examine the organization of the 5 S rRNA in relation to the 23 S rRNA and to determine whether it is in some way distinguishable from the organization of 23 S rDNA (fig.4). The identical pattern obtained indicated that both rRNA genes are included in the same fragments, suggesting a complete linkage of all 5 S rRNA genes to the 23 S rRNA genes.

The hybridization data are summarized in table 1, and we tried to fit the available results (figs 3–5) in a physical map (fig.6). It is concluded that the overall length of the combined structural genes will not be more than 5.35 kb; assuming a similar length for each structural gene, as was reported for *E. coli*, then the combined spacer region cannot exceed 0.8 kb.

From our *Bam*HI/*Hin*dIII hybridization data (figs 3,4) we have to place a *Hin*dIII site in the 23 S rRNA gene around position 1300. We conclude

Table 1

Summary of hybridization data

Restriction enzyme fragment	Ribosomal RNA species hybridized		
	5 S rRNA	23 S rRNA	16 S rRNA
*Bam*HI	6.0	6.0	6.0
	5.6	5.6	5.6
	4.8	4.8	4.8
	4.55	4.55	4.55
			19.0
			9.5
			6.6
			3.75
*Hin*dIII	>23.1	>23.1	2.45
	20.0	20.0	
	6.7	6.7	
	0.6	0.6	
*Bam*HI/*Hin*dIII	3.2	3.2	1.25
	2.8	2.8	1.20
	2.0	2.0	
	1.75	1.75	
		0.6	
*Bam*HI/*Eco*RI	4.75	4.75	1.1
	4.4	4.4	0.9
	3.6	3.6	
	3.3	3.3	
*Hin*dIII/*Eco*RI	7.25	7.25	2.0
	5.25	5.25	
	4.0	4.0	
	2.3	2.3	

The sizes (in kilobases) of DNA fragments hybridized to rRNAs or rDNA were determined by co-electrophoresis of *Hin*dIII fragments of lambda DNA

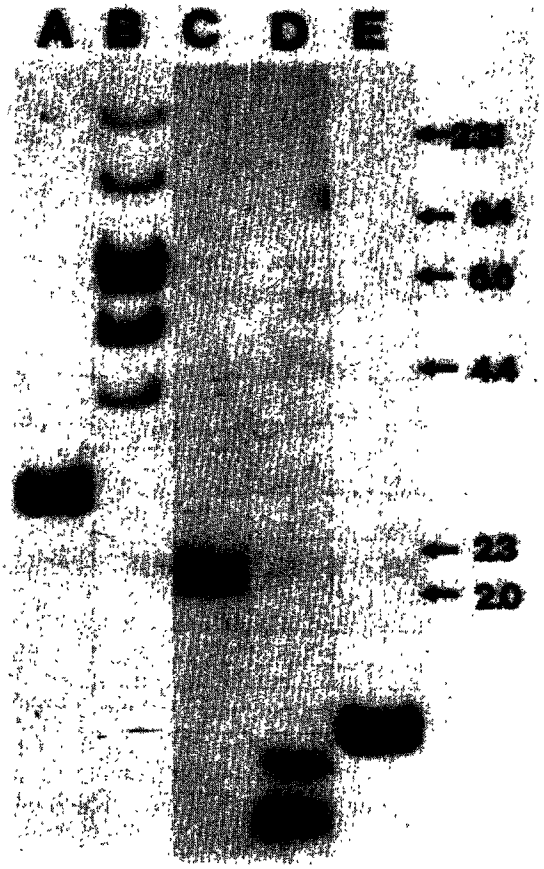

Fig.5. Autoradiographs of *P. aeruginosa* DNA fragments containing 16 S rRNA genes. The endonuclease-digested DNA were hybridized to nick-translated 16 S rDNA (pKK16). The molecular size of the reference is shown on the right of the column. Single digestion: (A) *Hin*dIII, (B) *Bam*HI; double digestion: (C) *Hin*dIII-*Eco*RI, (D) *Bam*HI-*Eco*RI, (E) *Bam*HI-*Hin*dIII. The band in lane E most probably consists of 2 bands, 1.25 and 1.20 kb, of nearly identical size (see also fig.2, lane B and fig.6).

that there are 2 further *Hin*dIII sites surrounding each 16 S rRNA gene (represented by the 2.45 kb fragment in fig.5), one located upstream of the 16 S rDNA coding region and one in or nearby the 16 S/23 S rDNA spacer region (fig.6). A *Bam*HI site within the 2.45 kb *Hin*dIII fragment (figs 2,5) leads to the formation of two 16 S rDNA *Bam*HI/*Hin*dIII fragments of almost identical size (putatively 1.2 and 1.25 kb).

The *Hin*dIII hybridization pattern for 23 S rRNA and 5 S rRNA (figs 3,4) is somewhat unsatisfactory because of the signal displayed at >23.1 kb. This situation, however, is clarified by the combined *Hin*dIII/*Eco*RI digests (figs 3,4), exhibiting 4 distinct hybridization bands as well as the 23 S rDNA internal 0.6 kb *Hin*dIII fragment mentioned before. Therefore, it is evident that the *Hin*dIII > 23.1 kb signal represents 2 indistinguishable rDNA fragments, each containing 23 S rDNA and 5 S rDNA coding sequences.

The 16 S rDNA has to be positioned in the *Eco*RI 2.0 kb fragment and the 23 S rDNA in the *Eco*RI/*Bam*HI digests, i.e. the 4.55, 4.8, 5.6 and 6.0 kb fragments, although we would like to propose that the maximal length of the structural genes including the terminators is 5.75 kb. In *E. coli* the promoters (P1, P2) have been located approx. 200 bp upstream from the 16 S rDNA, if this is the case for *P. aeruginosa* too, promoters can be expected in the *Hin*dIII/*Bam*HI, 1.2 kb fragment, or maybe even in the *Eco*RI/*Bam*HI, 0.9 kb, fragment.

From the combined data in fig.6 there will be a distance of 100–700 bp between the 16 S rDNA and 23 S rDNA coding sequences.

It is concluded that the chromosome of *P. aeruginosa* carries at least 4 sets of genes, each containing the genes for 16 S, 23 S and 5 S rRNA. There is no evidence for tandemly repeated 5 S rRNA genes. Each 5 S rRNA gene is closely linked to the 23 S rRNA genes and the number of transcriptional units of the rRNAs is in all probability 4.

This constellation is perfectly illustrated by the

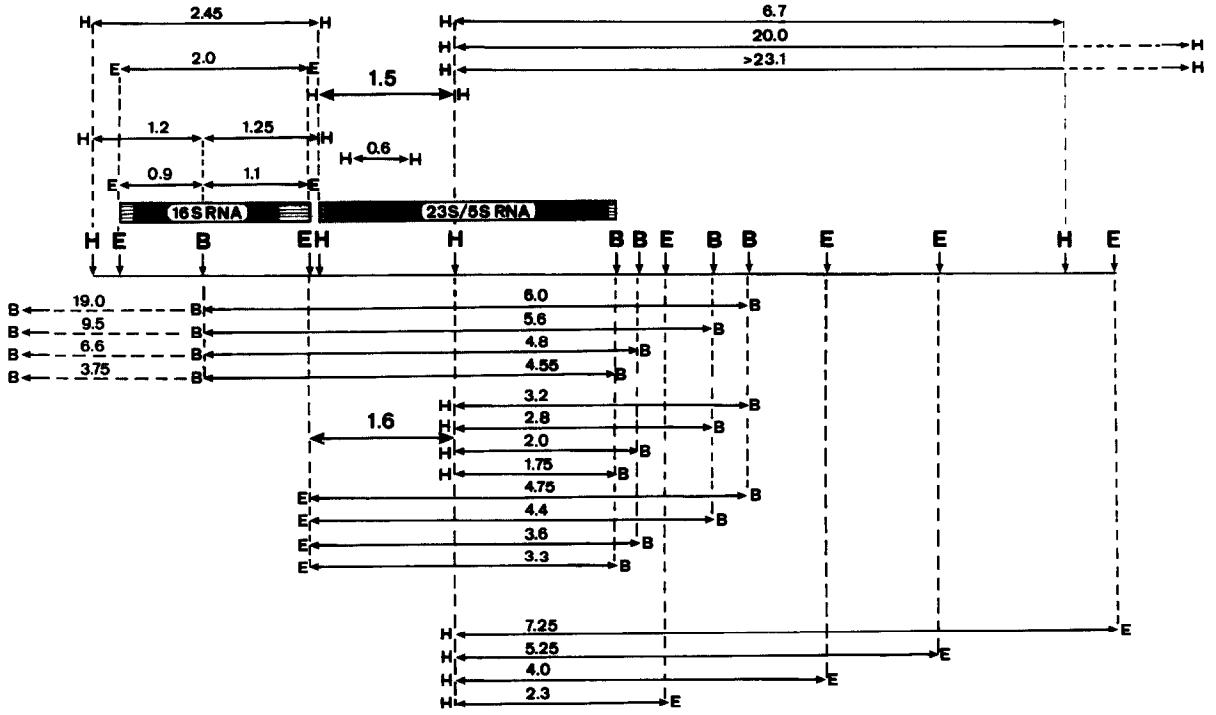

Fig.6. Proposed restriction pattern of *P. aeruginosa* rDNA operons. The schematic representation expresses the genomic layout of all rDNA operons. The restriction endonucleases employed are designated as follows: E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII.

*Bam*HI data (figs 3–5) yielding 4 fragments hybridizing with 16 S, 23 S and 5 S rDNA and 4 additional fragments which hybridize exclusively with 16 S rDNA.

The existence of many regions of the chromosome bounded by inverted repeats indicates configurations that are at least topologically equivalent to transposons [25] and suggests that a major avenue for the generation of duplicate genes in *E. coli* and other bacteria including pseudomonads has been via transposition of genetic material from one region to another, with retention of one copy at the original location. Thus, it is very likely that a similar situation for the appearance of multiple gene copies of rRNA operons exists in *Pseudomonas*.

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