Restriction Site Polymorphism of Ribosomal Ribonucleic Acid Gene Sets in Members of the Genus Bacillus

PAUL GOTTLIEB† AND RIVKA RUDNER*

Department of Biological Sciences, Hunter College of the City University of New York, New York, New York 10021

Hybridization of cloned ribosomal sequences to EcoRI-restricted genomic deoxyribonucleic acids of eight species and strains of the genus Bacillus produced multiband patterns consistent with the presence of 9 to 11 operons per genome. The basic structure of the repeating ribosomal gene set is highly conserved with the exception of one internal EcoRI site located near the abutment region between the 16S and 23S ribosomal ribonucleic acid determinants. In each of the Bacillus species studied, there are two abutment regions that differ in size by 0.2 kilobase; the larger region contains genes for isoleucine and alanine transfer ribonucleic acids at one-third the proportion of the smaller region. The occurrence of the EcoRI site in strains of Bacillus subtilis and Bacillus licheniformis gave rise upon cleavage to 1.2- and 1.4-kilobase abutment families. The absence of the EcoRI site in Bacillus globigii, Bacillus pumilus, and Bacillus amyloliquefaciens resulted in the emergence of 1.9- and 2.1-kilobase abutment families. Mixtures of the two types of families were not found in the Bacillus genomes studied.

Taxonomic relationships among various species of Bacillus have been studied by deoxyribonucleic acid (DNA)-DNA hybridization and by testing the transformability of auxotrophic and antibiotic resistance genetic markers (10, 21). We analyzed the degree of conservation and variation within ribosomal operons of members of the genus Bacillus by following the distribution of internal restriction sites in chromosome digests. The number and physical map of ribosomal ribonucleic acid (rRNA) gene sets in the genome of Bacillus subtilis strain 168M have been reported by workers from several laboratories (9, 15, 25; K. Bott, G. C. Stewart, and A. G. Anderson, in Syntro Conference on Genetics and Biotechnology of Bacilli, in press). There are 10 rRNA gene sets; each set comprises 16S, 23S, and 5S rRNA determinants, in that order, and two or three also contain genes for isoleucine and alanine transfer ribonucleic acids (tRNA^{ile} and tRNA^{aln}) (9). Unlike the situation in Escherichia coli, in which seven rRNA genes are scattered around the map (14), there is a unique clustering of the 10 gene sets in three groups in B. subtilis. The major cluster, which is composed of six or seven rRNA genes, is found in the chromosome segment delineated by purA and purB (Bott et al., in press; Rudner et al., unpublished data). Six gene sets have been mapped, rrnO and rrnR at the replication origin (15, 28), the closely situated repeats (rrnL, rrnH, and rrnG) near the attachment site of SPO2 (Bott et al., in press), and at least one (rrnE) between dal and purB (Rudner et al., unpublished data). The minor clusters containing three or four of the rRNA cistrons are located in the late replicating portion of the Bacillus genome. Operons rrnB and rrnC have been mapped in the region between thr-5 and argO (Bott et al., in press). At least one or two cistrons are believed to be located at the ilvBC-leu region (3, 23; P. Gottlieb, G. LaFauci, and R. Rudner, Gene, in press).

The basic physical map which is homologous in all gene sets of B. subtilis consists of three internal EcoRI and SmaI sites, two PstI sites, and single BamHI and HindIII sites (Fig. 1) (9, 25). The only restriction site which has been found to be variable is the HindIII site distal to the 5S rRNA determinant (Fig. 1) (25). Chromosomal patterns after restriction with EcoRI and BamHI have been used to calculate the number of gene sets in B. subtilis 168M (15, 25). The sizes of the various restriction fragments carrying ribosomal sequences vary considerably, indicating extensive heterogeneity in the inter-operon "spacer." To date, only one case of intragenic size heterogeneity has been reported, one which arises from the abutment region between the 3' terminus of 16S rRNA and the 5' terminus of 23S rRNA. There are two EcoRI-generated abutment fragments, measuring 1.1 and 1.3 kilobases (kb) according to Stewart et al. (25) or 1.2 and 1.4 kb according to our determinations and those of Loughney et al. (9). The larger spacer includes the genes for both tRNA^{ile} and tRNA^{aln} (9). We describe in this paper divergence among the Bacillus species involving sequences in the spacer region between the segment that transcribes the mature 16S and the 23S ribonucleic acid species.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used in this study are listed in Table 1. The plasmids containing ribosomal insertions were gifts from K. Bott and were amplified in Escherichia coli strain HB101 (pro leu thi lacY hsdR endA recA rpsL20 ara-14 galK2 xyl-5 mtl1 supE44). Figure 1 shows the sequences of genes coding for rRNA (rDNA) which were inserted into the plasmids used in this study. The cloned fragments originated as follows: (i) p21C4 contains a sheared 0.3-kb fragment around the EcoRI site in the 16S determinant cloned by adenine-thymine tailing into plasmid pMB9 (7); (ii) pBC194 contains a 1.1-kb EcoRI fragment with the abutment region of the 16S and 23S rDNAs and was subcloned from plasmid p14B1 into the EcoRI site of plasmid pBR322 (25); (iii) p12E2 contains a 6.3-kb BamHI-generated insertion with homology to 23S and 5S rDNAs and the 3.5-kb spacer DNA of the rrn operons (28) cloned into plasmid pBR313 by Zuber and Steinberg; (iv) pBC279 contains a 2.5-kb EcoRI-generated insertion with homology to 23S and 5S rDNAs cloned into plasmid pBR313 by Zuber and Steinberg; and (v) pMS102-B7 contains a 5.7-kb BamHI insertion which originated from rrnO (15) containing all of the restriction sites of interest.
the 16S rDNA and one-third of the 23S rDNA cloned into bifunctional plasmid pMS102 (20).

Chromosomal and plasmid DNA isolation and restriction patterns. Bacillus DNAs were prepared from cells grown in VY medium (25 g of veal infusion broth [Difco Laboratories], 5 g of yeast extract [Difco], and 1,000 ml of water) and were purified by a modification (19) of the procedure of Marmur (13). Plasmid DNA was purified from 500-ml cultures of Escherichia coli cells grown in LB broth supplemented with either 100 μg of ampicillin per ml or 15 μg of tetracycline per ml, essentially by the procedure of Tanaka and Weisblum (27). Chromosomal DNA (5 to 10 μg) was digested with restriction endonucleases EcoRI, HindIII, Smal, PstI, and BamHI (3 U of enzyme per μg of DNA for 12 h at 37°C) by using the conditions for digestion recommended by the supplier (New England BioLabs). DNA fragments were electrophoretically resolved in 0.75% agarose gels, stained with ethidium bromide, and transferred to nitrocellulose filters for hybridization (24).

Preparation of hybridization probes and densitometry. rRNA for use as a probe was isolated from B. subtilis 168T grown in VY medium and purified by the method of Margulies et al. (12); 5S, 16S, and 23S rRNA species were prepared in low-melting-point agarose (Bethesda Research Laboratories), and the rRNAs were 5' end labeled with [α-32P]adenosine triphosphate and T4 polynucleotide kinase by the method of Maizels (11). Alternatively, cloned rDNA and leu probes were prepared by nick translation with [α-32P]cytidine triphosphate (18). The labeled DNA was freed from low-molecular-weight material by passage through a Sephadex G-50 column (Pharmacia Fine Chemicals, Inc.). DNA was routinely labeled to a specific activity of 0.5 × 10^6 to 2 × 10^8 cpm/μg. Filter hybridization was performed by using the conditions of Ostapchuk et al. (16). The sizes of the bands obtained by autoradiography were determined by reference to six lambda bands and were calculated from the relationship described by Bearden (2). Some autoradiogram films were scanned at 580 nm with a Gilford automatic spectrophotometer.

FIG. 1. Generalized restriction map of B. subtilis rRNA gene sets as proposed by Stewart et al. (25). The six rRNA fragments from B. subtilis inserts into plasmids are represented by solid lines; the dashed lines represent B. subtilis DNA sequences which carry no homology to rRNA. B. BamHI; H, HindIII; S, Smal; R, EcoRI. The internal EcoRI site marked with a circled R is variable in other Bacillus species. The cross-hatched area defines the spacer or abutment region between 16S and 23S rRNA determinants. Sizes are given in kilobases.
equipped with a model 6051 recorder. The relative areas of the peaks were determined by using a planimeter (Noris Instruments).

**RESULTS**

Chromosomal patterns of rRNA homology in the genus *Bacillus*. Complete restriction of *Bacillus* DNAs with EcoRI, when they were probed with either 23S and 5S rRNAs or plasmids containing these sequences (pBC279 and p12E2), consistently produced 9 to 11 homologs on Southern blots. Figure 2 shows a composite of four autoradiograms obtained with EcoRI digests of *Bacillus* DNAs. Table 2 summarizes the molecular sizes and numbers of chromosomal restriction fragments which hybridized to the various probes found in each of the eight members of the genus *Bacillus* studied. As shown in Fig. 2, the multiple-band patterns were highly intense, indicating, as expected, that ribosomal sequences are extremely homologous in members of the genus *Bacillus* compared with other *Bacillus* sequences, such as the aromatic amino acid cluster (26) or the thrA gene (Rudner et al., unpublished data), which gave rise to hybrid bands of relatively low intensities. Similarly, other enzymes (data not shown), such as HindIII, BamHI, Smal, and PstI, revealed that the probes hybridized with equal intensity to all *Bacillus* DNAs. The different multiple-band hybridization patterns with respect to homolog size clearly indicate variation not in the rDNA sequences but in the spacer regions. The lower bands resulted from multiple restriction sites internal to the repeating gene set (see below) (Table 3). The upper bands arose from one restriction site within the gene set and a second site beyond it in the intergenic spacer region, where evolutionary variation has produced fragments of different sizes.

The multiple-band patterns obtained were unique for each of the species and were highly reproducible between individual strains when data were available. For *B. subtilis*, many derivatives of strains 168M and NCTC 3610 were analyzed; only two are shown in Table 2 and Fig. 2, in which the band patterns are identical. Not counting the last three hybrid bands, 9, 10, or 11 rRNA gene sets were identified by using the specific probes shown in Fig. 1. When EcoRI was used to cut *B. subtilis* DNA, only p21C4-16S, pBC194-16S,23S, and pMS102-16S,23S and 23S rRNAs hybridized to the 1.2- and 1.4-kb fragments (Fig. 2A and B) whereas 5S rRNA and pBC279-23S,5S and p12E2-23S,5S DNA probes did not hybridize to these fragments (Fig. 2C and D). The 0.9-kb fragment extended from the EcoRI site at the 5' end of the 23S coding sequence to the 16S coding sequence.

Polymorphism of the abutment fragments in *Bacillus*. The spacer or abutment fragments which overlap the 3' end of the 16S rRNA sequences and the 5' end of the 23S rRNA sequences were identified by using the specific probes shown in Fig. 1. When EcoRI was used to cut *B. subtilis* DNA, only p21C4-16S, pBC194-16S,23S, and pMS102-16S,23S and 23S rRNAs hybridized to the 1.2- and 1.4-kb fragments (Fig. 2A and B) whereas 5S rRNA and pBC279-23S,5S and p12E2-23S,5S DNA probes did not hybridize to these fragments (Fig. 2C and D). The 0.9-kb fragment extended from the EcoRI site at the 5' end of the 23S coding sequence to the 16S coding sequence. Therefore, it could hybridize with the comparable 23S determinants provided by plasmid probe p12E2-23S,5S (Fig. 2D). Table 3 shows the sizes of only these internal fragments and the hybridization probes that produced them. The two abutment fragments with similar hybridization patterns (that is, differing in size by 0.2 kb) were also obtained when Smal was used (see below). Our findings, as well as those reported previously (9, 25), indicate that there are two families of rRNA gene sets in *B. subtilis*, which differ with respect to the size of the EcoRI fragments bridging the 16S and 23S rRNA determinants. The intensity of the 1.4-kb band shown in Fig. 2A and B indicates that this fragment, like its 1.2-kb counterpart, exists in multiple copies at a ratio of 3:7, as reported by Stewart et al. (25), and at a similar relative proportion of 0.31 according to our densitometry tracing of the autoradiograms (see below).

Among the five *Bacillus* species and their strains examined in this study, the same pattern of internal EcoRI fragments was observed in *B. subtilis* strains NCTC 3610, 168M, and W23 and *B. licheniformis* strain ACTC 8480 (Fig. 2 and Table 3). However, in the case of *Bacillus globigii* RUB562, *Bacillus pumilus* RUB502, and *Bacillus amyloliquefaciens* H, the EcoRI site (Fig. 1, circled R) within the 5' end of the 23S sequence was not present. In these three species, the absence of that EcoRI site resulted in larger abutment fragments (1.9 to 2.1 kb) and the loss of the 0.9-kb EcoRI fragment. The p12E2-23S,5S probe revealed the 2.1- and 1.9-kb abutment fragments in the species that lost the EcoRI site (Table 3). However, in the species that retained the site (*B. subtilis* and *B. licheniformis*) (Fig. 2C). The loss of the internal EcoRI site resulted in extending the size of abutment fragments into the range of the p12E2-23S,5S probe (Fig. 1). Plasmid pBC279-23S,5S contains in its cloned sequence 23S rDNA extending only to the central EcoRI site (Fig. 1). As a result, neither the *B. subtilis* strains nor the *B. licheniformis* strains produced
FIG. 2. Hybridization of 32P-labeled cloned rDNA to EcoRI digests of Bacillus DNAs after Southern (24) transfer. Each gel lane contained 2 µg of restricted chromosomal DNA. The probes were prepared by nick translation, and the resulting specific activities ranged from $0.5 \times 10^6$ to $2 \times 10^6$ cpm/µg of DNA. The hybridization procedures of Anilionis and Riley (1) involved prehybridization of the filters in a solution containing 40% formamide, 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50 mM sodium phosphate (pH 6.5), 250 µg of sonicated calf thymus DNA per ml, 1% glycine, and 0.05 volume of Denhardt solution at 42°C for at least 1 h. The hybridizations with the 32P-labeled probes were for 18 to 22 h at 37°C in a similar solution in the presence of 10% dextran sulfate at a volume ratio of 40 ml/cm² of filter with digested chromosomal DNAs. (A) Autoradiogram of EcoRI-digested chromosomal DNAs probed with p21C4-16S. Lane 1, B. subtilis 168T; lane 2, B. amyloliquefaciens H; lane 3, B. globigii RUB562; lane 4, B. pumilus RUB502; lane 5, B. licheniformis FDO1; lane 6, B. licheniformis ACTC 8480. (B) Autoradiogram of EcoRI-digested chromosomal DNAs probed with pMS102-16S,23S. Lane 1, B. licheniformis FDO1; lane 2, B. licheniformis ACTC 8480; lane 3, B. pumilus RUB502; lane 4, B. globigii RUB562; lane 5, B. subtilis W23; lane 6, B. subtilis 168T; lane 7, B. subtilis NCTC 3610. (C) Autoradiogram of EcoRI-digested chromosomal DNAs probed with p12E2-23S,5S. Lane 1, B. licheniformis FDO1; lane 2, B. licheniformis ACTC 8480; lane 3, B. pumilus RUB502; lane 4, B. globigii RUB562; lane 5, B. subtilis W23; lane 6, B. amyloliquefaciens H; lane 7, B. subtilis GSY1269 repE26 ilvCl. (D) Autoradiogram of EcoRI-digested chromosomal DNAs probed with pBC179-23S,5S. Lane 1, B. licheniformis FDO1; lane 2, B. licheniformis ACTC 8480; lane 3, B. pumilus RUB502; lane 4, B. globigii RUB562; lane 5, B. subtilis W23; lane 6, B. subtilis NCTC 3610; lane 7, B. subtilis 168T; lane 8, lambda DNA, HindIII digested. kbp, kilobase pairs.
their abutment region as this probe shares no homology with it (Fig. 1 and 2D). Plasmid pMS102-16S,23S, as well as the end-labeled 23s rRNA probe, showed the three internal pieces (Fig. 2B).

Additional heterogeneity in internal fragment size was observed in two Bacillus strains. One of these was B. licheniformis strain FDO1, which produced an extra abutment fragment when it was probed with plasmid pBC194-16S,23S or pMS102-16S,23S. As shown in Fig. 5, a 1.3-kb band was found in addition to the two abutment fragments (1.2 and 1.4 kb). The ratio of the 1.4-kb band to the sum of the 1.2- and 1.3-kb bands in strain FDO1 was 0.45, which was significantly higher than the value of 0.28 found for the 1.4- and 1.2-kb abutment fragments in strain ACTC 8480. As noted above, B. licheniformis strain FDO1 has 11 rrn gene sets (Table 2). The additional operon might contain an insertion of a single tRNA in its abutment region, giving rise to the B. subtilis DNA probes containing parts of tRNA$^{16\text{c}}$ or tRNA$^{5\text{db}}$ (9).

A similar situation was observed in the EcoRI digests of B. amyloliquefaciens strain H. Here the digests, when they were probed with pBC194, produced a doublet at the position of the 1.9-kb band and two minor bands (Fig. 5). The latter corresponded to the 1.4- and 1.2-kb bands found in the first group of Bacillus species (Table 3). Finally, Bacillus steatotherophilus strain 2184 gave rise to a single internal EcoRI fragment (1.9 kb) (Fig. 5).

**DISCUSSION**

In this paper we present data on the conserved nature of the ribosomal gene sets among Bacillus species. The basic rrn repeating unit is highly conserved, whereas extensive heterogeneity is found in the interoperon spacer regions. In chromosomal restrictions, B. subtilis strain NCTC 3610 is identical to strain 168M. In contrast, these two strains differ significantly from prototrophic strain W23, which was once used as a source of donor DNA for transformation. B.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Strain(s)</th>
<th>Fragment sizes (kb) with the following probes:</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoRI</td>
<td><em>B. subtilis</em> 168T, NCTC 3610, and W23</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>W23</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td><em>B. licheniformis</em> FDO1 and NCTC 8480</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td><em>B. globigii</em> RUB562</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td><em>B. pumilus</em> RUB502</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td><em>B. amyloliquefaciens</em> H</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.9</td>
</tr>
<tr>
<td>SmaI</td>
<td><em>B. subtilis</em> 168T, NCTC 3610, and W23</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>W23</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>B. globigii</em> RUB562</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td><em>B. pumilus</em> RUB502</td>
<td>—</td>
</tr>
</tbody>
</table>

* — Hybrid band not present.
ND, Not determined.

*B. licheniformis* FDO1 exhibited three hybrid bands (1.4, 1.3, and 1.2 kb) (see Fig. 5).

*B. amyloliquefaciens* H exhibited two additional faint hybrid bands (1.4 and 1.2 kb) (see Fig. 5).

### FIG. 3. Densitometry tracing of an autoradiogram of EcoRI 23S rRNA homologs.

Chromosomal DNA of *B. subtilis* NCTC 3610 was restricted to completion with endonuclease EcoRI (3 U of enzyme per µg of DNA). Then 2 µg of the digest was added to each well of a 0.75% agarose gel. Southern blots were made of these gels and hybridized to 32P-labeled 23S rRNA (30 to 40 ng of probe per gel lane). The hybridization reaction was for 12 h under the conditions described by Ostapchuk, et al. (16). The hybridized filter was filmed for 10 h at room temperature. The chart paper of the densitometer recorder was run at 20 cm/min, and the autoradiogram lane was scanned at 4 cm/min (580 nm). The numbers over the peaks are molecular sizes (in kilobases).
13, and 7% for *B. amyloliquifaciens* F, *B. licheniformis* (13 strains), and *B. pumilus* IFO 12110, respectively (21). Based on their DNA homology index data, Seki et al. divided the genus *Bacillus* into three groups. One of these groups had a homology index to strain 168M of more than 70% and transformed widely dispersed auxotrophic markers. The second group, with a level of DNA homology to strain 168 of 20 to 25%, did not transform auxotrophic markers but did transform antibiotic resistance. The third group had a level of DNA homology to strain 168 of 7 to 15% and showed some detectable transformation of antibiotic resistance markers (21).

The *Bacillus* strains examined in this study can be divided into two groups, those with the internal EcoRI site (type I) and those without this site (type II). Upon digestion with restriction enzymes EcoRI and Smal, the DNAs of these strains yielded abutment fragments differing in size by precisely 0.2 kb. With the exception of one strain, none of the strains gave rise to a mixture of both types of fragments (namely, a 1.4-kb fragment and a 1.9-kb fragment or a 1.2-kb fragment and a 2.1-kb fragment). In *B. amyloliquifaciens* H we observed small amounts of the lower bands (1.4 and 1.2 kb), yet more than 80% of the hybrids were the two larger abutment fragments (Fig. 4). Moreover, we predict no variation in the distribution of abutment fragments in *Bacillus* DNAs digested with restriction enzyme PstI or in a double digest with PstI and HindIII. The sizes expected for the conserved abutment regions are 3.1 and 3.3 kb for the former enzyme and 0.9 and 1.1 kb for the codigestion (9). The absence of additional heterogeneity in fragment size strongly suggests that the original loss of that EcoRI site which led to the appearance of the two larger abutment fragments (1.9 and 2.1 kb) could have occurred in a proto-*Bacillus* species before the main rRNA gene amplification. Interoperon spacer variations, genomic rearrangements, and gene duplication evolved later and led to the observed changes in the molecular sizes of the larger rDNA fragments. An evolution-
FIG. 6. Dendrogram analysis of the relationships among five species of Bacillus and their strains. The bacteria examined in this study were divided into two lines of descent based upon the structures of their rRNA gene sets. Type I Bacillus species have rRNA genes possessing the 5' 23S sequence EcoRI site (see Fig. 1), and the type II species have rRNA genes without the EcoRI site.

ary dendrogram which illustrates a proposed derivation and relationship among these Bacillus species is shown in Fig. 6. Alternatively, horizontal evolution via amplification of one variant rrn cistron could also produce this result. Unequal crossing-over can fix a given sequence type within a population of heterogeneous sequences (22). According to this model, an entire gene cluster is replaced by a different type of gene copy, an event that would occur after rrn gene multiplication. Smith (22) has presented a model of “cross-over fixation,” which could effectively propagate a variant sequence with the elimination of another. This event also depends upon the occurrence of unequal crossing-over among tandem repeats of genes in a tandem array of a fixed size; through such unequal crossing-over the original genes are eventually eliminated and replaced by others. Such a model allows the fixation for type I or type II rDNA sequences to occur at any time in evolution and clearly explains how a gene variant originally in the minority can ultimately become the predominant or only type. In fact, we describe in another study mutants of B. subtilis displaying an rDNA deletion or duplication as evidence that such a mechanism might be occurring (Gottlieb et al., in press). The addition, alteration, or replacement of rDNA in transformants of these mutants lends further credence to the dynamic nature of the rDNA repeats (Gottlieb et al., in press; Rudner et al., unpublished data).

In summary, the physical organization of an rRNA gene set in the genus Bacillus is unique in size, as shown by the positions of restriction site sequences which remained unchanged. It is intriguing that even the length of the spacer regions between 16S and 23S rRNA coding sequences, which potentially could have varied, are highly conserved. Only two sizes have evolved, differing by 0.2 kb; the larger abutment fragment contains the tRNA^{asn} and tRNA^{alu} genes (9). Similar tRNA sequences are located in the analogous region of the Escherichia coli rRNA operons (29), as well as in chloroplastic rRNAs of Euglena gracilis (5), and Zea mays (8). This strong sequence homology between spacer regions of gram-positive bacteria and gram-negative bacteria, as well as the chloroplasts of eucaryotes, indicates functional significance and structural constraints. It is tempting to speculate that the spacer regions, particularly those with the tRNA genes, can form secondary structures which constitute part of a signal structure necessary for proper processing of the common precursors. On the other hand, the intergenic spacer region between adjacent rRNA operons seems to have suffered a higher genetic drift rate in the genus Bacillus.

ACKNOWLEDGMENTS

We thank the following students for assisting in some of these studies: D. Nembhard, R. Sepulveda, and W. Williamson. This work was supported by Public Health Service grant GM16059 from the National Institute of General Medical Sciences, by City University of New York Faculty Research Awards 14024 and 663152, and by Public Health Service Minority Biomedical
Research Support Grant RRO81776-04 from the National Institutes of Health.

**ADDENDUM IN PROOF**

K. F. Bott (University of North Carolina Medical School, Chapel Hill) has made available to us the complete sequence of the *B. subtilis rrrB* operon which confirms the size of the abutment EcoRI fragments as 1.1 or 1.3 kb.

**LITERATURE CITED**