Structure and organization of the rrnD operon of 'Brevibacterium lactofermentum': analysis of the 16S rRNA gene

Eladio Amador, José M. Castro, Antonio Correia and Juan F. Martin

Five rRNA operons (rrn) were found by hybridization in the genome of 'Brevibacterium lactofermentum' ATCC 13869 and Corynebacterium glutamicum ATCC 13032. 'B. lactofermentum' DSM 20412 differed from the other corynebacteria tested in showing six hybridizing BamHI bands. Two of the rrn operons (rrnD and rrnE) were located in a single cosmid. Sequencing of the rrnD operon showed that it contains a complete 16S rRNA-23S rRNA-5S rRNA gene cluster. Phylogenetic studies using the complete 16S rRNA sequence showed that 'B. lactofermentum' is closely related to several species of the genus Corynebacterium but only distantly related to the type species Brevibacterium linens and the authors suggest that it should be reclassified as Corynebacterium lactofermentum. The 5' end of mature 16S rRNA was identified by primer extension. Sequence elements similar to those of mycobacteria implicated in transcription antitermination (Boxes A, B, C) and in processing of the pre-rRNA to 16s rRNA were identified. An open reading frame encoding an rpoD-like sigma factor (named SigC) different from the previously reported SigA and SigB proteins was found upstream of rrnD in the opposite orientation. Both rpoD and sigC seem to be expressed from a bidirectional promoter region.

Keywords: corynebacteria, rRNA, clusters, expression, sigma factor

INTRODUCTION

'Brevibacterium lactofermentum' belongs to the 'glutamic acid' group of non-pathogenic corynebacteria, so called because of their well-known amino acid overproducing capability (Eikmanns et al., 1993). Knowledge on the molecular biology of this group of bacteria has advanced considerably in the last decade, including studies on codon usage (Malumbres et al., 1993), sigma factors (Oguiza et al., 1996), promoters (Pátek et al., 1996), terminators and regulatory sequences (Martin, 1989; Guerrero et al., 1994; Mateos et al., 1994), insertion sequences (Bonamy et al., 1994; Correia et al., 1996; Vertes et al., 1994b), transposon mutagenesis (Vertes et al., 1994a) and genome organization (Bathe et al., 1996; Correia et al., 1994). Several amino acids are accumulated extracellularly at millimolar levels in cultures of 'B. lactofermentum' or Corynebacterium glutamicum following growth limitations that impair protein synthesis. However, in spite of their industrial importance little is known about the molecular mechanisms that control growth and amino acid production (Malumbres & Martin, 1996).

The control of rRNA and protein synthesis is a complex and strictly regulated process; the first level of this regulation occurs at the initiation of transcription of rRNA and is a key to understanding the control of ribosome synthesis and, therefore, to elucidating growth control and amino-acid producing ability.

rRNA (rrn) operons are transcribed as single units giving pre-rRNAs, which are cleaved by RNases to pre-16S rRNA, pre-23S rRNA and pre-5S rRNA. Further RNase processing produces mature rRNA species. There are numerous bacterial rRNA sequences available (mainly used for phylogenetic studies), including some partial sequences from corynebacteria deposited in GenBank; however, no information was available on the upstream regulatory sequences or about transcription of rrn operons in corynebacteria. It was, therefore, of great interest to study the rrn operons of amino acid
producing corynebacteria as a way to understand growth regulation and the mechanism that triggers amino acid accumulation under nutritional starvation.

C. glutamicum and ‘B. lactofermentum’ were initially proposed to belong to two different genera (Yamada & Komagata, 1970; Abbe & Takayama, 1972). ‘B. lactofermentum’ was separated from other Brevisbacterium species by its ability to ferment lactose, producing acid in litmus milk (Abbe & Takayama, 1972). However, Liebl et al. (1991) proposed to transfer ‘B. lactofermentum’ to the genus Corynebacterium, as C. glutamicum. The cloning of the rRNA operons (rrn) provides a very useful instrument to settle the polemic about the taxonomic relatedness of these two glutamic acid producing corynebacteria. In this article we describe the characterization of an rRNA operon from ‘B. lactofermentum’, including the complete sequence of the 16S rRNA gene and upstream regulatory region and a transcription analysis. Analysis of the rrn operons of C. glutamicum and ‘B. lactofermentum’ shows that these two species are closely related but not identical.

METHODS

Bacterial strains, plasmids and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. Escherichia coli strains were grown at 37°C on Luria-Bertani medium supplemented with ampicillin (50 μg ml⁻¹) when needed. Corynebacteria were grown on trypticase soy broth (TSB) or trypticase soy agar (TSA) (trypticase soy broth with 2% agar).

DNA hybridization. Chromosomal DNA from corynebacterial strains was digested with BamHI and EcoRI. DNA fragments were resolved by electrophoresis and transferred to nylon membranes by standard methods (Sambrook et al., 1989). Specific probes were labelled with [³²P]dCTP (Amersham) by nick-translation (Promega). Blots were hybridized at high stringency (50% formamide, 42 °C) as described by Sambrook et al. (1989).

DNA sequencing. Subclones of the cloned DNA fragments were sequenced on both strands by the dideoxynucleotide chain-termination method (Sanger et al., 1977) using a Pharmacia ALF automatic sequencer.

RESULTS

Corynebacterial rRNA gene clusters: cloning of a ‘B. lactofermentum’ ribosomal operon

Total DNA from (i) ‘B. lactofermentum’ ATCC 13869 and two derivatives (R31 and a recA mutant), (ii) ‘B. lactofermentum’ DSM 20412 and (iii) C. glutamicum

Table 1. Strains and plasmids used

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description*</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>Bacteria</td>
<td></td>
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<tr>
<td>E. coli XL1Blue</td>
<td>Standard strain for DNA manipulations</td>
<td>Stratagene</td>
</tr>
<tr>
<td>E. coli WK6mutS</td>
<td>Δ[lac-proAB] galE strA mucS215 Tn10/F lacIZΔM15 proA*</td>
<td>R. Zell, Jena, Germany</td>
</tr>
<tr>
<td>‘B. lactofermentum’ ATCC 13869</td>
<td>Type strain</td>
<td>ATCC†</td>
</tr>
<tr>
<td>‘B. lactofermentum’ R31</td>
<td>Melys’ Acc’; restriction-deficient mutant of ATCC 13869</td>
<td>Santamaria et al. (1985)</td>
</tr>
<tr>
<td>‘B. lactofermentum’ recA</td>
<td>Recombination-deficient mutant of ATCC 13869</td>
<td>K. Dunican, University of Galway, Ireland</td>
</tr>
<tr>
<td>‘B. lactofermentum’ DSM 20412</td>
<td></td>
<td>Liebl et al. (1991)</td>
</tr>
<tr>
<td>C. glutamicum ATCC 13032</td>
<td>Type strain</td>
<td>ATCC†</td>
</tr>
<tr>
<td>Plasmid, phage and cosmid</td>
<td></td>
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</tr>
<tr>
<td>M13K07</td>
<td>Helper phage</td>
<td>Mead &amp; Kemper (1988)</td>
</tr>
<tr>
<td>pBluescript KS(+)</td>
<td>Phagemid derived from pUC; Ap′</td>
<td>Stratagene</td>
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<tr>
<td>pBA2</td>
<td>Plasmid with an insert of 16S rRNA from Bacillus subtilis</td>
<td>Iglesias et al. (1983)</td>
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* Melys’, resistance to N-methyllysine; Acc’, resistance to S-aminoethylcysteine.
† American Type Culture Collection.
**Fig. 1.** *B. lactofermentum* rRNA gene clusters. Southern blots of BamHI (left) or EcoRI (right) digests were hybridized with a 2.3 kb HindIII DNA fragment from pBA2 encoding the 16S rRNA of *Bacillus subtilis*. Strains used were: Lanes 1 and 6, *B. lactofermentum* DSM 20412; lanes 2 and 7, *C. glutamicum* ATCC 13032; lanes 3 and 8, *B. lactofermentum* ATCC 13869; lanes 4 and 9, *B. lactofermentum* recA; lanes 5 and 10, *B. lactofermentum* R31. Sizes of the bands for ATCC 13869 are indicated on the left.

ATCC 13032 was digested with BamHI, EcoRI and other enzymes (see below) and probed with a 2.3 kb HindIII fragment from pBA2 (Iglesias et al., 1983) containing part of a *Bacillus subtilis* ribosomal operon. Five BamHI hybridizing bands were observed for all strains used except one, *B. lactofermentum* DSM 20412 (see below), with sizes of 14.5, 10.7, 8.9, 8.7 and 8.0 kb for *B. lactofermentum* ATCC 13869 except for the slowest-migrating band, which showed a smaller size than the corresponding bands of *B. lactofermentum* ATCC strains. Therefore, we cannot exclude the possibility that the sixth BamHI band in *B. lactofermentum* DSM 20412 is originated by an internal BamHI site in one of the *rrn* operons that does not occur in the other *B. lactofermentum* strains.

When the *B. lactofermentum* ATCC 13869 cosmid bank (Correia et al., 1996) was probed with the *Bacillus subtilis* 2.3 kb HindIII fragment, 60 out of 480 recombinant cosmids gave a positive hybridization signal. Twenty of them were selected and digested with BamHI and the bands were blotted and reprobed. Some of the cosmids contained two positive BamHI bands, of 8.0 and 8.7 kb, indicating that at least two of the five operons are linked in a 40 kb DNA region (data not shown), an arrangement that also occurs in other bacteria. Bathe et al. (1996) reported a scattered location of four *rrn* loci in the *C. glutamicum* chromosome but admitted that one of the *rrn* loci may contain two *rrn* clusters.

Assuming that each of the five BamHI genomic bands of *B. lactofermentum* ATCC 13869 that gave a positive signal contains an *rrn* operon we designated them by letters A to E in decreasing size order; the *rrn* operons located in the 8.7 and 8.0 kb bands subcloned from the same cosmid (see Fig. 2) corresponded to the hybridizing bands D and E, respectively. A region of approximately 5.5 kb internal to both *rrnD* and *rrnE* operons is boxed

**Fig. 2.** Restriction map of two *B. lactofermentum* rRNA operons. BamHI fragments of 8.7 kb (*rrnD*) and 8.0 kb (*rrnE*) from the same cosmid were cloned in pBluescript. The regions outlined with a dotted box show a similar restriction pattern. A, Apal; B, BamHI; Bs, BstXI; C, ClaI; E, EcoRI; Ev, EcoRV; H, HindIII; N, NotI; S, SalI; Sc, SacI; Sp, SpeI; P, PstI; X, XbaI; Xh, XhoI. The three arrows correspond to the 16S, 23S and 5S genes found in *rrnD*. The 1.2 kb BamHI–Kpnl fragment containing the upstream region of operon *rrnD* and the ORF1 is indicated by a thin line below. The 27 kb probe of *Bacillus subtilis* is indicated by a solid bar.
Fig. 3. Model of the secondary structure of 'B. lactofermentum' 16S rRNA based on that of E. coli (Noller, 1984) and M. tuberculosis (Kempsell et al., 1992). The domains and motifs in the sequence are indicated. V1, V2, etc., are variable regions.
rRNA operons of 'Brevibacterium lactofermentum'

**Fig. 4.** Phylogenetic tree of 'B. lactofermentum' and some other Gram-positive bacteria (particularly coryneform bacteria) according to 16S RNA sequence. The tree was constructed using the CLUSTAL method (MegAlign, DNASTAR). Accession numbers: 'C. fastidiosum' X84245, 'C. segmentosum' X84437, 'C. accolens' X80500; C. pseudotuberculosis D38577, C. afermentans X81874, C. renale X81909, C. mycetoides X84241, C. cystitidis X84252, C. diphteriae X84248, C. ammoniagenes X84440, C. striatum X81910, C. variabilis X53185, C. glutamicum X84257, C. callunae X84251, C. xerosis X84446, M. bovis X55589, M. tuberculosis X52917, M. leprae X53999, M. gordonae X52923, R. fascians X53204, B. linens X77451, Frankia sp. U72717, S. hygroscopicus X79853, Bacillus subtilis X60646, 'B. lactofermentum' Y12792 (B. Brevibacterium; C., Corynebacterium; M., Mycobacterium; R., Rhodococcus; S., Streptomyces). Note the low degree of homology between B. linens (the type species of the genus Brevibacterium) and 'B. lactofermentum'.

**Box B**

| B. lactofermentum | CCCGAAATGGG | TGTTTAAAGGC | AACTAAAGTG | GTGTTGAGG |
| M. tuberculosis   | CCCGAAATGGG | TGTTTAAAGGC | AACTAAAGTG | GTGTTGAGG |

**Table 1.** Alignment of the antiterminator motifs (Boxes B, C and A) and the RNase III cleavage site of M. tuberculosis with similar motifs of 'B. lactofermentum'. Similar motifs occur in all mycobacteria and other Gram-positive bacteria. The similarity is given as percentage of identical nucleotides in the motif.

in Fig. 2. Band D was confirmed to contain one rRNA operon by total sequencing of the 16S gene and partial sequencing of the 23S and 5S genes and comparison with known rRNA sequences deposited in GenBank. Analysis of these subclones confirmed the 16S–23S–5S gene arrangement present in the majority of bacteria. No evidence of the presence of any tRNA gene in the gene cluster was obtained.

**Primary and secondary structure of the 16S rRNA**

A 2.7 kb HindIII fragment as well as a 1.2 kb BamHI–KpnI fragment from the rrnD operon were subcloned in pBluescript SK(+) (both orientations) and used for sequencing the entire 16S RNA gene and the 5’ regulatory region. The putative 5’ end of the mature 16S rRNA was assigned at nucleotide position 1171 numbered from the left BamHI site of the sequence and the 3’ end was located at nucleotide position 2664 by comparison with the 16S rRNA genes of Mycobacterium spp. (Ji et al., 1994a, b, c) and Streptomyces coelicolor (Condon et al., 1995). Therefore, the 'B. lactofermentum' 16S rRNA gene is 1494 nt long, slightly larger than those described for Streptomyces or Mycobacterium species.

The anti-Shine–Dalgarno sequence comprising a tract of 91 nucleotides was found to be present immediately upstream of the ribosome-binding site.
10 pyrimidines with the sequence 5' CCUCCUUUCC at the 3' end of the 'B. lactofermentum' 16S rRNA showed total identity with the sequence described for Mycobacterium tuberculosis by Kempsell et al. (1992); therefore, the hypothetical Shine–Dalgarno mRNA sequence complementary to the anti-Shine–Dalgarno of 'B. lactofermentum' is 5' AGAAAGGAGG (see Fig. 3).

Since the sequences of bacterial 16S rRNAs are highly conserved they have been used for phylogenetic studies. When the sequence for structural 16S rRNA was compared with those present in the GenBank/EMBL databases an optimal alignment was found with other corynebacterial sequences. The homology was in the range 90–96% when compared with all sequences belonging to the genus Corynebacterium (95.6% with C. glutamicum); however, the homology was significantly lower (82%) with Brevibacterium linens ATCC 9172, the type species of the genus. Fig. 4 shows a phylogenetic tree of some representative eubacteria with special reference to corynebacteria (see Discussion).

rRNAs are known to fold into several stem and loop structures by internal pairing. The 'B. lactofermentum' 16S rRNA sequence was aligned and folded into a secondary structure (Fig. 3) based on the folding model for rRNAs proposed by Noller (1984) for E. coli. The secondary structure obtained for 'B. lactofermentum' is highly similar to that observed in Mycobacterium tuberculosis (Kempsell et al., 1992). The sequence conservation is higher in loop regions than in paired regions and only some motifs are different, especially in helix 5 and 6 (region V1). The sequence of helix 5 is a characteristic feature of a particular 16S rRNA (Ji et al., 1994a).

Also significant differences were seen in the variable regions V2 (helix 8 and 10), V3 (helix 18) and V8 (helix 43 and 44) as expected. The remaining differences were mainly nucleotide substitutions.
Transcription of the 'B. lactofermentum' rrnD operon and processing of the transcript

The 5' end of mature 16S rRNA and sequence elements implicated in processing of the pre-rRNA to form 16S rRNA were identified by primer extension and by comparison with elements with the same function recognized in a wide range of bacteria (King et al., 1986). Antitermination of transcription occurs in the E. coli rRNA operons; therefore, we searched for possible antitermination elements present in the leader region of rRNA similar to those described for species of Mycobacterium (González-y-Merhand et al., 1996; Ji et al., 1994a, b). Three elements similar to Box B, Box A and Box C from M. tuberculosis were identified with 83.3, 87.5 and 77.7% identical nucleotides respectively (Fig. 5). Three nucleotides upstream of Box B there is a G (position 758 in Fig. 6) that is a putative site for the transcription initiation of the pre-rRNA that also occurs in M. tuberculosis.

Pre-16S rRNA derives from pre-rRNA by the action of RNase III. One RNase III cleavage site was located within the leader region (sequence 5' AACTAA) showing total identity with the cleavage site described in Streptomyces coelicolor and Mycobacterium species. In addition, a palindromic, presumably forming a loop around the RNase III site (AACAACTAACC TTATGGTT) was found. A GC-rich sequence known as the discriminator, close to the +1 RNA transcription start site, has been noted as a common feature of promoters regulated by the stringent response (Condon et al., 1995). A putative sequence with such a function (GGTGGCCC) was found partially overlapping Box B.

Two oligonucleotides, O-1 and O-2 (in bold face in Fig. 6), were used to locate by primer extension the RNA 5' ends resulting from either transcription initiation or processing in the relatively short sequence between the nucleotide +1 corresponding to the mature 16S rRNA and the upstream open reading frame. Results of the primer extension are shown in Fig. 7. Four signals were observed: two strong ones corresponding to consecutive Gs (nucleotides 1169 and 1170 in Fig. 6) appeared repeatedly using oligonucleotide O-2 and two very faint ones were observed with O-1 as primer. The strong signals correspond to the processing sites for the 16S mature rRNA. Sites for transcription initiation and processing are, therefore, located in a short stretch of 500 nt upstream of the sites that we found by primer extension and the upstream open reading frame. Preliminary evidence indicates that a short DNA fragment upstream of Box B showed promoter activity when coupled to a promoter probe vector (E. Amador, J. M. Castro & J. F. Martin, unpublished results).

An rpoD-like sigma factor gene is located upstream of rrnD

Upstream from the Box B motif of the rrnD operon, in the same DNA strand but in the opposite orientation, ORF1 (224 residues) was identified starting at nucleotide 672 with a GTG codon that is truncated in the initial BamHI site (Fig. 6). Computer analysis gave a significant homology with SigA sigma factor of 'B. lactofermentum' described by Oguiza et al. (1996) (Fig. 8). The protein encoded by ORF1 has an 87% amino acid identity with SigA specially in a region of 110 amino acids corresponding to the 1.1 domain of rpoD-like sigma factors. The homology with SigB was much lower since SigB does not contain the 1.1 region. We have named this rpoD-like gene sigC.

DISCUSSION

There is a broad correlation between the number of ribosomes and the growth rate of different bacteria (Bremer & Dennis, 1987). The number of ribosomes in the cell depends on the availability of ribosomal proteins and rRNA, which is determined by the number of rRNA operons, the strength of their promoters and the efficiency with which the operons are transcribed.

Five rRNA gene clusters have been found in 'B. lactofermentum' ATCC 13869. There are seven rRNA operons in E. coli (Condon et al., 1992), six in Streptomyces coelicolor (van Wezel et al., 1994), four in Streptomyces ambofaciens (Pernodet et al., 1989), two in Myco-

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**Fig. 8.** Alignment of the deduced amino acid sequence of ORF1 with the sequence of SigA of 'B. lactofermentum'. Regions 1.1 and 1.2 of rpoD-like (sigma-70) factors are overlined. Amino acids which are identical in both sequences are shown in reverse typeface.
bacterium phlei and Mycobacterium smegmatis (Bercovier et al., 1986; Ji et al., 1994c) but only one in the slow-growing Mycobacterium tuberculosis and Mycobacterium leprae (Bercovier et al., 1986). Corynebacteria of the 'glutamic acid' group contain more rrr operons than mycobacteria, which is consistent with their faster growth rate.

Sequencing of the 16S rRNA has allowed us to identify an anti-Shine–Dalgarno sequence for corynebacteria. There were no previous studies about the degree of complementarity between the sequence of the 3' end of the 16S rRNA and the sequence of the mRNA 5' to the translational start codon (Martin, 1989). In a few cases the putative Shine–Dalgarno sequence is located 2–10 nt (2–11 nt in E. coli) upstream from the AUG or GUG start codon and has the potential of forming four to eight base-pairs with the 3' end of 16S rRNA. E. coli has a shorter anti-Shine–Dalgarno sequence (consensus AGGA). Sequences that show good complementarity to the anti-Shine–Dalgarno sequence described in this article have been found preceding the coding sequences of many genes cloned from corynebacteria (see review by Martin, 1989).

Analysis of the rrrD sequence has provided information on the phylogeny of 'B. lactofermentum' and on the molecular control of macromolecule gene expression.

There has been controversy about the relationship between the two corynebacteria used routinely as amino acid producing strains, 'B. lactofermentum' and C. glutamicum (Liebl et al., 1991; Correia et al., 1994). Phylogenetic studies of coryneform bacteria by analysis of 5S rRNA sequences suggested that they should be clustered together with Nocardia, Streptomyces and Micrococcus species and indicated that Brevibacterium species are phylogenetically distant from other coryneform bacteria (Park et al., 1987). Liebl et al. (1991) proposed to transfer 'B. lactofermentum' to the genus Corynebacterium (as C. glutamicum) based on DNA–DNA hybridization, chemotaxonomic and biochemical studies. However, a restriction fragment length polymorphism analysis in which oligonucleotides targeted against conserved regions of 16S and 235 rRNA genes were used as probes showed that although there is a relatively high degree of similarity there were enough clear differences to allow separation of 'B. lactofermentum' from C. glutamicum. The overall restriction map as shown by PFGE (Correia et al., 1994) showed significant differences that confirmed earlier reports on the difference between these two organisms based on their morphological characteristics.

One conclusion of our 16S rRNA analysis is that B. lactofermentum ATCC 13869 should no longer be considered as a member of the genus Brevibacterium and that it should be transferred to the genus Corynebacterium, in agreement with the proposal of Liebl et al. (1991). However, the divergence shown in Fig. 4 between 'B. lactofermentum' and C. glutamicum is higher than that between different species of the genus Coryne-

bacterium, e.g. C. fastidiosum, C. segmentosum, C. accolens and C. pseudotuberculosis, which indicates that 'B. lactofermentum' is a different species of the genus Corynebacterium. We suggest, therefore, that 'B. lactofermentum' should be changed to Corynebacterium lactofermentum, maintaining the name of the species. 'B. lactofermentum' was clearly different from the glutamic acid producer C. ammoniagenes (Fig. 4). It would be interesting to study if similar conclusions apply to other glutamic acid producing corynebacteria such as 'C. melassecola' and 'B. flavum', but no data are available on their rrr genes.

Four rpoD-like genes were found by hybridization with an rpoD probe in the genome of 'B. lactofermentum'. Two of these genes, sigA and sigB, were sequenced (Oguiza et al., 1996). ORF1, extending upstream from rrrD, encodes a gene similar to but different from sigA and sigB, that has been named sigC. Although this gene is truncated in the cloned fragment, the presence of 1.1 and part of the 1.2 regions of rpoD-like genes (Lonetto et al., 1992) suggests that the gene encoded by ORF1 is indeed an additional sig gene.

The presence of an rpoD-like gene in the opposite orientation to that of rrrD that must be expressed from a promoter located in the bidirectional promoter region is intriguing. Clustering of genes for macromolecule (rRNA) biosynthesis and sigma factors suggests that a common control mechanism (e.g. stringent control: Condon et al., 1995; Wehmeier et al., 1998) exerted on the bidirectional promoter region may affect sigma factor formation and rRNA synthesis.

The two strong signals found by primer extension differed by one nucleotide and correspond to the 5' end of the mature 16S rRNA. However we could not clearly observe the 5' end corresponding to the pre-16S rRNA although the RNase cleavage sequence AACUAA identical to that of mycobacteria and Bacillus subtilis (Ji et al., 1994a, c; Ogasawara et al., 1983) is conserved in the upstream region. It is likely that processing of pre-rRNA and pre-16S into 16S rRNA is very efficient and therefore we cannot detect the pre-rRNA. The similarity of elements A, B and C present in the non-coding region of rrrD with those of mycobacteria suggests that an antitermination mechanism of control of transcription similar to that reported in other species (Berg et al., 1989; Ji et al., 1994a) also occurs in corynebacteria. In E. coli the Box A sequence of the pre-rRNA forms a complex with the transcriptional factor nusB and protein S10, which then attach to the RNA polymerase, allowing transcription to proceed (Nodwell & Greenblatt, 1993).

ACKNOWLEDGEMENTS

This work was supported by grants from the European Union (BIO4-CT96-0145), the CICYT, Madrid (BIO92-0708), and Junta de Castilla y León (LE02/96, LE18/96 and LE29/93). E. Amador received a fellowship of the University of León, and A. Correia was supported by a fellowship of the Science
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Received 3 September 1998; revised 21 December 1998; accepted 4 January 1999.