JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4855–4864
0021-9193/88/104855-10$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 170, No. 10

Cloning and Characterization of *Bacillus subtilis* Homologs of *Escherichia coli* Cell Division Genes *ftsZ* and *ftsA*

BERNARD BEALL, MICHAEL LOWE, AND JOE LUTKENHAUS*

Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas 66103

Received 7 June 1988/Accepted 20 July 1988

The *Bacillus subtilis* homolog of the *Escherichia coli ftsZ* gene was isolated by screening a *B. subtilis* genomic library with anti-*E. coli* FtsZ antiserum. DNA sequence analysis of a 4-kilobase region revealed three open reading frames. One of these coded for a protein that was about 50% homologous to the *E. coli* FtsZ protein. The open reading frame just upstream of *ftsZ* coded for a protein that was 34% homologous to the *E. coli* FtsA protein. The open reading frames flanking these two *B. subtilis* genes showed no relationship to those found in *E. coli*. Expression of the *B. subtilis ftsZ* and *ftsA* genes in *E. coli* was lethal, since neither of these genes could be cloned on plasmid vectors unless promoter sequences were first removed. Cloning the *B. subtilis ftsZ* gene under the control of the *lac* promoter resulted in an IPTG^s phenotype that could be suppressed by overproduction of *E. coli* FtsZ. These genes mapped at 135° on the *B. subtilis* genetic map near previously identified cell division mutations.

The process of cell division in *Escherichia coli* requires at least 10 genes (7). Little is known about the biochemical function of the corresponding gene products, although many have been cloned and their gene products have been identified. The *ftsZ* gene appears to play a pivotal role in the cell division process, since overexpression leads to a hyperdivision activity displayed as the minicell phenotype (34) and the FtsZ protein is the target of the SOS-induced division inhibitor, SulA (SfiA) (14, 17).

Because of its apparent pivotal role in cell division, it is of interest to determine to what extent the gene has been conserved during evolution. Recently, it was reported that many, if not all, gram-negative and gram-positive bacteria contain a polypeptide that cross-reacts with antisera against *E. coli* FtsZ protein (5). To determine the extent of sequence and functional conservation, it is necessary to clone the gene from bacteria of other genera. In addition, the *ftsZ* gene in *E. coli* lies within a large cluster of genes required for normal cell wall physiology (7, 18), and it will be of interest to determine to what extent this organization has been retained during evolution.

It seemed of greatest interest to clone the gene from *Bacillus subtilis* for several reasons. First, *B. subtilis* is distantly related to *E. coli*, and many genes from the two organisms show various degrees of homology. Second, many cell division mutants of *B. subtilis* have been isolated (25), and it would be possible to determine whether any of these mutants carry mutations in a homolog of *ftsZ*. Such a finding would support functional conservation. Third, *B. subtilis* has an SOS-like response following DNA damage which resembles that found in *E. coli* (16). The response includes inhibition of cell division, and it is possible that the target of the *B. subtilis* SOS response is also FtsZ. Fourth, during sporulation of *B. subtilis* an asymmetric septum is formed that has unique features that differentiate it from the vegetative septum (11). It is therefore intriguing to determine whether the FtsZ protein plays any role in vegetative cell division in *B. subtilis* and to see to what extent it may contribute to the unique septation occurring during sporulation.

* Corresponding author.

In this report we present the isolation, mapping, and initial characterization of the *B. subtilis* homologs of the *E. coli* cell division genes *ftsZ* and *ftsA*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this work are listed in Table 1. *B. subtilis* auxotrophs and Tn*917* insertion strains used for mapping of the *ftsZ-ftsA* region were obtained from the *B. subtilis* Genetic Stock Center at Ohio State University. *B. subtilis* BD224 (8), which carries the *recE4* allele, was used for cloning the *B. subtilis ftsZ* (*ftsZ*$_{Bs}$) gene onto multicopy shuttle vector plasmids. *E. coli* strains JM101 (32) and JFL101 (F′::Tn*9 lacI*^{q}) (18) were used to assess the effects of *ftsZ*$_{Bs}$ gene expression on *E. coli*. Strains JM101 and JFL100 (F′::Tn*9 lacI*^{q}) (34) were used for observing cell morphology after induction of the *ftsA*$_{Bs}$ gene. *E. coli* GM161 was used as a host to obtain DNA that was deficient in methylated adenine residues.

Plasmid and phage constructions. An M13 derivative, mp19Z1, containing the *ftsZ*$_{Bs}$ gene was obtained by ligating the 2.3-kilobase (kb) *Eco*RV-*Bgl*II fragment from λBS1 into M13mp19 (19) digested with *Hin*cII and *Bam*HI. Since this same fragment could not be cloned on a plasmid in *E. coli*, it was cloned on a plasmid in *B. subtilis*. To do this, advantage was taken of two shuttle vectors. Plasmid pLI50, obtained from Chia Lee, contains the pUB110 and pBR322 origins of replication, the pC194 CM^r determinant, and the Amp^r gene of pBR322. This plasmid also contains the polylinker fragment of M13mp18 (19). The *ftsZ*$_{Bs}$ gene was cloned into pLI50 by ligating *Sph*I-*Eco*RI-digested mp19Z1 and pLI50 and transforming competent BD224 cells to CM^r. Plasmids from these transformants were screened for the presence of the insert. One such plasmid was designated pBZ1. This plasmid could not be transformed into *E. coli* due to expression of the *ftsZ*$_{Bs}$ gene. Two derivatives of pBZ1 were constructed in which the *ftsZ*$_{Bs}$ gene was inactivated. In the first, pBZ2, the orientation of a 400-base-pair (bp) *Asu*II fragment at the 5′ end of the *ftsZ*$_{Bs}$ gene was reversed. In a second, pBZ1Δ*Asu*II, this 400-bp fragment was deleted, which resulted in an in-frame deletion. Both of these derivatives were selected by simply transforming *E. coli* JFL101 with ligated *Asu*II digests of pBZ1.

TABLE 1. Bacterial strains used in this study

Strain	Genotype	Source or reference
Bacillus subtilis		
168	*str*	I. Goldberg
BD224	*recE4 thr-5 trpC2*	8
IA5	*glyB133 metC3 tre-12 trpC2*	6
IA607 (SPβc2)	*metC85*::Tn*917 trpC2*	31
IA609 (SPβc2)	*pyr-82*::Tn*917 trpC2*	31
IA610 (SPβc2)	*pyr-83*::Tn*917 trpC2*	31
Escherichia coli		
JM101	Δ(*lac-proAB*)/F′ *traD36 proAB*	32
JFLL101	*ftsZ84 recA ilv deo ara*(Am) *lacZ125*(Am) *galU42*(Am) *trp*(Am) *tyrT supFA81*(Ts)	18
JFL100	*ftsA12*(Ts) *thr leu thi pyrF thyA ilvA his arg lac tonA tsx*	18
GM161	*dam-4 thr-1 leuB6 thi-1 hsdS1 lacY1 tonA21 supE44*	B. Bachmann

The intact *ftsZ*$_{Bs}$ gene was cloned into *E. coli* by removing upstream sequences that presumably contained a promoter(s). Plasmid pBZ1-1 was derived by *Bal*31 digestion of *Sph*I-digested pBZ1, followed by ligation in the presence of excess *Bam*HI linker. This plasmid was selected by transformation into *E. coli* JFL101 and was found to contain a deletion extending from the *Sph*I site to approximately 30 bp upstream of the *ftsZ*$_{Bs}$ Shine-Dalgarno sequence. The *ftsZ*$_{Bs}$ gene was subsequently cloned downstream of the *lac* promoter in the shuttle vector pMK4 (28) by using the *Bam*HI and *Eco*RI sites. A pMK4 derivative containing the appropriate fragment was designated pMKZ-1. This plasmid was first obtained in *B. subtilis* but subsequently could be introduced into *E. coli* strains containing excess Lac repressor from the *lacI*^{q} determinant.

The *ftsA*$_{Bs}$ gene was cloned into M13 by ligating the 2.1-kb *Asu*II-*Hin*dIII fragment of λBS1 into *Acc*I-*Hin*dIII-digested M13mp19. The resultant clone, designated mpA1, contains the entire structural gene and about 1 kb of upstream sequence which includes a closely juxtapositioned vegetative promoter. A 1.4-kb *Taq*I fragment, which contains the *ftsA*$_{Bs}$ structural gene and just 9 bp upstream of the *ftsA*$_{Bs}$ Shine-Dalgarno sequence, was subsequently cloned into the *Acc*I site of pUC18 (19) in the correct orientation for expression from the *lac* promoter. This plasmid, designated pUCA1, was obtained by screening Amp^r transformants of JM101 for isopropyl-β-D-thiogalactopyranoside (IPTG)-induced filamentation on L-agar containing 1 mM IPTG.

Several additional plasmids were used. Plasmid pJH101 (10) is an integrational vector for *B. subtilis*. It consists of pBR322 with the addition of a Cm^r determinant which can be expressed in both *B. subtilis* and *E. coli*. The 1-kb *Hin*dIII fragment from λBS1, located downstream of the *ftsZ*$_{Bs}$ gene, was cloned into pJH101 to give pJH101B. This plasmid was used for mapping. Plasmid pBS58, which was constructed in this laboratory by Bharati Sanjanwala, consists of a 5-kb *Pst*I fragment containing the *E. coli* genes *ftsQ*, *ftsA*, and *ftsZ* cloned into the pSC101 derivative pGB2 (3).

Chromosomal mapping of the *ftsZ*$_{Bs}$-*ftsA*$_{Bs}$ region. Since pJH101B does not contain an origin of replication for *B. subtilis*, transformation of *B. subtilis* to Cm^r with this plasmid should result from homologous insertion of the plasmid into the *B. subtilis* chromosome. One such Cm^r transformant of *B. subtilis* 168 *str* was transduced to Ery^r by phage PBS1 grown on strains containing Tn*917* insertions at various chromosomal locations (31). The Ery^r transductants were then scored for loss of Cm^r to determine linkage of the two antibiotic resistance determinants. Similarly, PBS1 grown on Cm^r *B. subtilis* 168 *str* was used to transduce various auxotrophs to Cm^r, followed by scoring for auxotrophic markers.

DNA sequencing. DNA was sequenced by the dideoxy method with JM101 as host for M13mp18 and M13mp19 derivatives (19). Initially the Klenow fragment (Bethesda Research Laboratories) was used for sequencing, but later Sequenase (United States Biochemical Corporation) was preferred. In two instances additional synthetic primers were used to facilitate the sequencing of portions of the *ftsA*$_{Bs}$ and *ftsZ*$_{Bs}$ genes.

Immunoblotting. Proteins electrophoresed on sodium dodecyl sulfate (SDS)-polyacrylamide gels were transferred and immunostained as described previously with antisera against purified *E. coli* FtsZ (34).

Labeling plasmid-encoded proteins. Strain JFL101 (F′::Tn9 *lacI*^{q}) was infected with phage M13 derivatives or transformed with plasmids. Proteins were labeled by the maxicell system described by Sancar et al. (27). The labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and autoradiography.

RESULTS

Cloning the *ftsZ*$_{Bs}$ gene. A *B. subtilis* chromosomal DNA library constructed in λ Charon 4A (9) was screened for *ftsZ*$_{Bs}$ expression with antiserum prepared against purified *E. coli* FtsZ. Previously we have shown by Western (immunoblot) analysis that this antiserum cross-reacts with a single polypeptide from *B. subtilis* with a slightly slower mobility than *E. coli* FtsZ (5). Restriction mapping of five positive clones revealed that they all contained two *Eco*RI fragments of 2.8 and 11 kb (Fig. 1). To confirm that these clones contained the gene coding for the presumed *ftsZ*$_{Bs}$ gene, the phage were used to infect *E. coli*, and cell lysates prepared 30 min after infection were subjected to Western analysis. This analysis revealed, in addition to the resident *E. coli* FtsZ, the presence of an immunostaining band corresponding in mobility to that observed in cell lysates of *B. subtilis* (data not shown). Thus, it appeared that the entire *ftsZ*$_{Bs}$ gene was contained on these phage.

To try to locate the *ftsZ*$_{Bs}$ gene, various restriction fragments were subcloned into plasmid vectors and analyzed for expression of an immunoreactive band. The fragments that were subcloned, including the 2.8-kb *Eco*RI fragment and the 4.2-kb *Hin*dIII fragment, were negative. However, the 11-kb *Eco*RI fragment and the 3.3-kb *Hin*dIII fragment that is contained within this *Eco*RI fragment could not be cloned on a high-copy-number plasmid to test for expression. As an alternative strategy, we decided to obtain DNA sequence

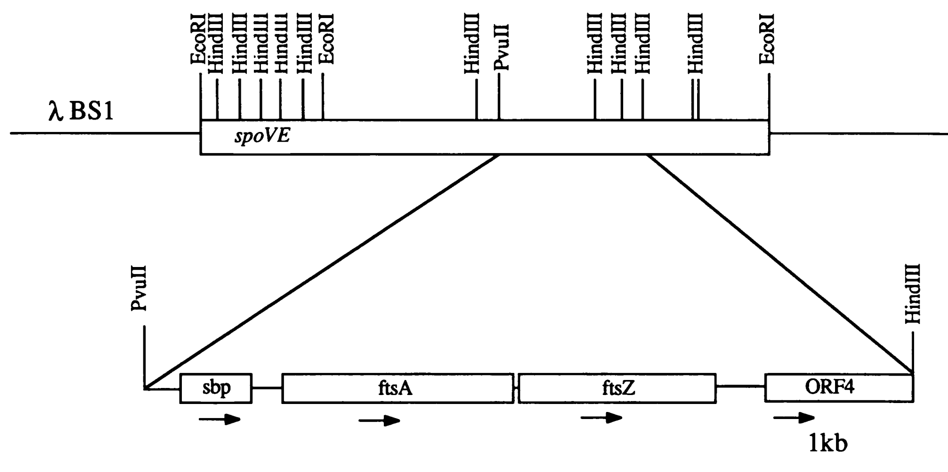


FIG. 1. Physical map of λBS1. Phage λBS1 contains two *Eco*RI fragments of 2.8 and 11 kb. The region expanded below the map was sequenced. The arrows indicate the orientation of the genes. Only the *Pvu*II site used in this study is indicated, and any additional sites have not been mapped. The location of the *spoVE* gene is taken from Piggot et al. (24).

information and see whether the $ftsZ_{Bs}$ gene could be localized by looking for homology of the deduced amino acid sequence to the *E. coli* FtsZ protein. Limited sequence analysis of portions of the 3.3-kb *Hin*dIII fragment indicated the presence of genes homologous to the *E. coli ftsZ* and *ftsA* genes.

DNA sequence analysis of the $ftsZ_{Bs}$ region. After we had localized the $ftsZ_{Bs}$ gene, the complete nucleotide sequence of a 4-kb region of DNA was determined that encompassed the $ftsZ_{Bs}$ and $ftsA_{Bs}$ genes and flanking DNA (Fig. 1). The nucleotide sequence and the deduced amino acid sequences are presented in Fig. 2. Three long open reading frames were found in this region, all with the same orientation, and all preceded by a strong ribosome-binding site, AGGAGG. The first open reading frame, starting with the ATG codon at positions 42 to 44, consisted of 121 codons and would code for a small basic protein with a molecular size of 13.3 kilodaltons (kDa) and an isoelectric point of 10.6. The second open reading frame, beginning with an ATG codon at positions 581 to 583, consisted of 440 codons and would code for a protein of 48.1 kDa and an isoelectric point of 5.9. This protein shared 34.3% amino acid identity with the FtsA protein of *E. coli* in a 380-amino-acid overlap (Fig. 3). The *B. subtilis* protein was larger due to a carboxy-terminal extension. The third open reading frame, beginning with an ATG codon at positions 1939 to 1941, consisted of 382 codons and would code for a protein of 40.3 kDa and an isoelectric point of 5.8. This protein had approximately 50% amino acid identity with the FtsZ protein of *E. coli* (Fig. 4). The homology was strongest in the first two-thirds of the sequence, 60% identity, whereas in the last one-third the homology was weaker, 24% identity. A possible fourth open reading frame started 300 bp downstream of $ftsZ_{Bs}$, beginning with an ATG at positions 3389 to 3391, and ran until the end of the fragment. This open reading frame was preceded by a run of five G's, which could act as a ribosome-binding site, and would code for a protein of greater than 20 kDa. No sizable open reading frames within this 4-kb region were detected on the opposite strand.

Figure 5 shows a comparison of the gene organization in the *ftsA-ftsZ* regions of *E. coli* and *B. subtilis*. The tandem arrangement of the two genes was preserved, although the flanking genes in *B. subtilis* showed no homology to the *ftsQ* and *envA* genes of *E. coli*.

Subcloning of the $ftsZ_{Bs}$ and $ftsA_{Bs}$ genes. Various attempts to clone restriction fragments containing either the $ftsZ_{Bs}$ or $ftsA_{Bs}$ gene or both into high-copy-number or relatively low-copy-number plasmids (8 to 10 copies per cell) were unsuccessful, suggesting that overproduction of these *B. subtilis* gene products may be lethal to *E. coli*. Surprisingly, we were able to clone these genes into derivatives of phage M13. A 2.1-kb *Hin*dIII-*Asu*II fragment, containing the entire $ftsA_{Bs}$ structural gene, was cloned into M13mp19, as was a 2.3-kb *Eco*RV-*Bgl*II fragment containing the entire $ftsZ_{Bs}$ structural gene (Fig. 6). In both cases the structural genes were oriented so that they could be expressed from the *lac* promoter on the phage. To determine whether the gene products were expressed, the phage were used to infect a *recA* mutant strain and proteins were labeled by the maxicell procedure or detected by Western analysis. When M13mp19 was labeled by this procedure, only one labeled band was visible (Fig. 7A, lane 3). With mpA1, which contains the $ftsA_{Bs}$ gene, an additional band with a molecular weight of 48 kDa was observed (Fig. 7A, lane 2). This size is consistent with the deduced size of the $FtsA_{Bs}$ gene product. For mpZ1, which contains $ftsZ_{Bs}$, an immunostained band in addition to *E. coli* FtsZ was observed; it was induced by IPTG and had a slightly slower mobility than *E. coli* FtsZ (Fig. 7B, lane 1). The Western blot also showed that even in the presence of IPTG, cells containing mpZ1 produced only a small amount of the protein (relative to the amount of *E. coli* FtsZ that was present). Presumably, this lack of efficient expression permitted cloning of the genes in this vector. Attempts to subclone the genes from these phage into plasmid vectors were unsuccessful, even in the absence of any external promoters.

As an alternative strategy, we decided to subclone the $ftsZ_{Bs}$ gene from mpZ1 directly into *B. subtilis*. The $ftsZ_{Bs}$ gene was cut out of mpZ1 with *Sph*I and *Eco*RI, which cut in the polylinker region, and cloned into the shuttle vector pLI50 by selecting for chloramphenicol resistance in *B. subtilis* and screening for the presence of the fragment (Fig. 6). The desired plasmid, designated pBZ1, was readily obtained. This plasmid was purified and used to transfect several *E. coli* strains; however, no transfectants were obtained. The strains that were used included strains that overproduce *E. coli* FtsZ to a small extent as well as strains that contain various alleles of *ftsZ*.

```
     PvuII
1    CAGCTGAAACTGCTTAAAGACTGAAAAGGAGGAGAATTGTC  ATG TGG CTG CCC
                                          (sbp) M   W   L   P

54   GTG TTG GGA CTG GTG CTC GGA ATA GCG ATT GGA CTA ATG ACA AAC
      V   L   G   L   V   I   A   I   G   L   M   T   M   T   N

99   TTA ACG ATA CCG AGT GAA TAC TCA AAC TAT TTA TCG CTT GCG GTG
      L   T   I   P   S   E   Y   S   N   Y   L   S   L   A   V

144  CTT GCC GCA CTT GAT ACA TTA ATC GGC GGA ATC AGG GCG CAT TTA
      L   A   A   L   D   T   L   I   G   G   I   R   A   H   L

189  CAA GGT ACA TAT GAT GAA ATG GTA TTT GTT TCC GGT TTC TTT TTT
      Q   G   T   Y   D   E   M   V   F   V   S   G   F   F   F

234  AAT ATT ATA TTG GCA ATA AGT TTA GCT TTT CTG GGA GTC CAT CTT
      N   I   I   L   A   I   S   L   A   F   L   G   V   H   L

279  GGT GTA GAC TTG TAT TTA GCA GGT ATA TTC GCA TTT GGA GTC AGA
      G   V   D   L   Y   L   A   G   I   F   A   F   G   V   R

324  TTA TTT CAG AAT ATA GCC GTT ATC AGA AGA AAT CTA CTA ACA AAG
      L   F   Q   N   I   A   V   I   R   R   N   L   L   T   K

389  TGG ACT CTT TCT AAA AAA AAT AAA AAA AAT GTG ATA TAA AAGAGGA
      W   T   L   S   K   K   N   K   K   N   V   I   -

415  TATACATAGGATATAACGAATATTTTCAATAAACATAAAATGTGAAAAGCACATAAAAA
                                                          -35
474  TATTCTGTTGTTATTTTTTGTTACACACTTGTAAAGCCACATTCATTGTATTGTTGTTC
             -10
533  CGCAAATAATAGAATAGAAATGATCGAAATGTGAGGAGGTGCCATAGA    ATG AAC
                                                  (ftsA) M   N

587  AAC AAT GAA CTT TAC GTC AGT CTT GAC CTC GGT ACG TCC AAT ACC
      N   N   E   L   Y   V   S   L   D   L   G   T   S   N   T

632  AAA GTG ATC GTC GGA GAA ATG ACA GGT GAT TCC CTT AAT ATT ATC
      K   V   I   V   G   E   M   T   G   D   S   L   N   I   I

677  GGT GTG GGA AAT GTA CCG TCT GAA GGG TTG AAA AAA GGC TCA ATC
      G   V   G   N   V   P   S   E   G   L   K   K   G   S   I

722  GTT GAT ATA GAT GAG ACC GTT CAT TCT ATA AGA AAA GCG TTT GAC
      V   D   I   D   E   T   V   H   S   I   R   K   A   F   D

767  CAA GCT GAA AGA ATG GTA GGT TTT CCG CTT AGA AAA GCT ATT GTC
      Q   A   E   R   M   V   G   F   P   L   R   K   A   I   V

812  GGC GTT AAT GGA AAT TAT ATC AAT ATT CAA GAT ACA AAC GGT GTT
      G   V   N   G   N   Y   I   N   I   Q   D   T   N   G   V

857  GTA GCA GTT TCC AGT GAA AAT AAA GAA ATT CAG GTT GAA GAT GTA
      V   A   V   S   S   E   N   K   E   I   Q   V   E   D   V

902  CGC CGT GTC ATG GCG GCG GCA CAG GTT GTT TCC GTA CCG CAT GAA
      R   R   V   M   A   A   A   Q   V   V   S   V   P   H   E
     PvuII
947  CAG CTG ATT GTT GAC GTC ATT CCT AAA CAG TTT ATC GTA GAT GGA
      Q   L   I   V   D   V   I   P   K   Q   F   I   V   D   G

992  AGA GAT GAA ATC ACC GAC CCG AAA AAA ATG CTG GGT GTG CGT TTA
      R   D   E   I   T   D   P   K   K   M   L   G   V   R   L

1037 GAA GTA GAG GGC ACC TTG ATC ACC GGT TCA AAA ACA ATC TTA CAT
      E   V   E   G   T   L   I   T   G   S   K   T   I   L   H

1082 AAC TTA CTC CGC TGC GTT GAA AGA GCT GGT ATT GAA ATA ACT GAT
      N   L   L   R   C   V   E   R   A   G   I   E   I   T   D

1127 ATT TGC CTT CAG CCG CTG GCA GCC GGT TCT GCT GCA TTA TCA AAG
      I   C   L   Q   P   L   A   A   G   S   A   A   L   S   K

1172 GAC GAG AAA AAC CTT GGT GTG GCT CTC ATT GAT ATA GGG GGA GGG
      D   E   K   N   L   G   V   A   L   I   D   I   G   G   G

1217 TCA ACA ACC ATT GCC GTA TTC CAG AAC GGA CAT CTC ACT TCT ACC
      S   T   T   I   A   V   F   Q   N   G   H   L   T   S   T
                                                  EcoRV
1262 CGT GTC ATT CCT CTC GGA GGC GAA AAT ATC ACC AAA GAT ATC TCC
      R   V   I   P   L   G   G   E   N   I   T   K   D   I   S

1307 ATC GGA TTG AGA ACG TCA ACA GAA GAA GCA GAG CGC GTG AAA AAG
      I   G   L   R   T   S   T   E   E   A   E   R   V   K   K

1352 CAA CTT GGA CAT GCC TAC TAT GAC GAA GCC TCG GAA GAT GAA ATA
      Q   L   G   H   A   Y   Y   D   E   A   S   E   D   E   I
                                                          -35
1397 TTT GAG GTA ACC GTT ATA GGC ACC AAT CAA AAA CAA ACA TTT ACA
      F   E   V   T   V   I   G   T   N   Q   K   Q   T   F   T
                             -10
1442 CAG CAA GAA GCA GCG AAT ATC ATT GAA GCG AGA GTA GAG GAA ATT
      Q   Q   E   A   A   N   I   I   E   A   R   V   E   E   I

1487 CTT GAA ATT GTT TCA GAA GAG CTT CGC AGT ATG GGA ATT ACC GAT
      L   E   I   V   S   E   E   L   R   S   M   G   I   T   D

1532 CTG CCG GGA GGC TTC GTG CTG ACA GGA GGA CAA GCA GCT ATG CCG
      L   P   G   G   F   V   L   T   G   G   Q   A   A   M   P

1577 GGT GTC ATG TCT TTG GCA CAG GAT GTG CTG CAA AAC AAT GTC AGA
      G   V   M   S   L   A   Q   D   V   L   Q   N   N   V   R

1622 GTG CAA GGC CCG AAT TAT ATC GGT GTA AGA GAT CCT CAA TAT ATG
      V   Q   G   P   N   Y   I   G   V   R   D   P   Q   Y   M

1667 ACG GGA GTG GGC CTG ATC CAA TTC GCC TGC CGG AAT GCA AGA ATC
      T   G   V   G   L   I   Q   F   A   C   R   N   A   R   I

1712 CAA GGC AGA AAA ATA GGC TTT AAG ATG CCT GAA GAA GCG ATA CAG
      Q   G   R   K   I   G   F   K   M   P   E   E   A   I   Q

1757 GAA ATC GCA GTC TCA TCA TCT GAG GAA CAA GAG CAG CAT CAT CAC
      E   I   A   V   S   S   S   E   E   Q   E   Q   H   H   H

1802 CAA AAT GAG GTG CAG CAG CGG CCA AAA GGA AAA CAA AAA ACA CAA
      Q   N   E   V   Q   Q   R   P   K   G   K   Q   K   T   Q

1847 GCC GAA CAT AAT AAA CAG AGC AAA ATG AAA AAA CTA TTA AGC ATG
      A   E   H   N   K   Q   S   K   M   K   K   L   L   S   M

1892 TTT TGG GAA TAG ATAGATAGTCATTCGGCAGATTAGGAGGATTTAGC ATG TTG
      F   W   E   -                                (ftsZ) M   L
         AsuII
1945 GAG TTC GAA ACA AAC ATA GAC GGC TTA GCA TCA ATT AAA GTA ATC
      E   F   E   T   N   I   D   G   L   A   S   I   K   V   I

1990 GGA GTA GGA GGC GGC GGT AAC AAC GCC GTT AAC CGA ATG ATT GAA
      G   V   G   G   G   G   N   N   A   V   N   R   M   I   E

2035 AAT GAA GTG CAA GGA GTA GAG TAT ATC GCG GTA AAC ACG GAC GCT
      N   E   V   Q   G   V   E   Y   I   A   V   N   T   D   A

2080 CAA GCT CTT AAC CTG TCA AAA GCA GAA GTG AAA ATG CAA ATC GGC
      Q   A   L   N   L   S   K   A   E   V   K   M   Q   I   G

2125 GCA AAG CTG ACT AGA GGA TTG GGA GCA GGT GCG AAT CCG GAA GTC
      A   K   L   T   R   G   L   G   A   G   A   N   P   E   V

2170 GGG AAA AAA GCC GCT GAA GAA AGC AAA GAG CAG ATT GAA GAA GCA
      G   K   K   A   A   E   E   S   K   E   Q   I   E   E   A
                                             PvuII
2215 CTT AAA GGT GCT GAC ATG GTA TTC GTG ACA GCT GGT ATG GGC GGC
      L   K   G   A   D   M   V   F   V   T   A   G   M   G   G

2260 GGA ACA GGA ACA GGT GCC GCA CCG GTT ATC GCA CAA ATC GCG AAA
      G   T   G   T   G   A   A   P   V   I   A   Q   I   A   K

2305 GAC TTA GGC GCA TTA ACA GTC GGC GTT GTG ACA AGA CCG TTT ACC
      D   L   G   A   L   T   V   G   V   V   T   R   P   F   T
     AsuII
2350 TTC GAA GGA CGC AAA AGA CAG CTT CAG GCT GCA GGC GGA ATC TCG
      F   E   G   R   K   R   Q   L   Q   A   A   G   G   I   S

2395 GCA ATG AAA GAA GCG GTG GAT ACA CTG ATC GTG ATC CCG AAC GAC
      A   M   K   E   A   V   D   T   L   I   V   I   P   N   D

2440 CGT ATC CTT GAA ATT GTT GAT AAA AAC ACA CCG ATG CTT GAA GCA
      R   I   L   E   I   V   D   K   N   T   P   M   L   E   A

2485 TTC CGC GAA GCG GAT AAC GTA CTT CGC CAA GGG GTT CAA GGT ATT
      F   R   E   A   D   N   V   L   R   Q   G   V   Q   G   I

2530 TCT GAC TTG ATT GCT ACA CCT GGT CTT ATC AAC CTT GAC TTT GCT
      S   D   L   I   A   T   P   G   L   I   N   L   D   F   A

2575 GAT GTG AAA ACA ATC ATG TCA AAC AAA GGA TCT GCT TTG ATG GGT
      D   V   K   T   I   M   S   N   K   G   S   A   L   M   G

2620 ATC GGT ATT GCT ACT GGG GAA AAT CGC GCG GCA GAG GCA GCA AAA
      I   G   I   A   T   G   E   N   R   A   A   E   A   A   K

2665 AAA GCA ATT TCC AGC CCG CTT CTT GAA GCG GCC ATT GAC GGT GCG
      K   A   I   S   S   P   L   L   E   A   A   I   D   G   A

2710 CAA GGC GTC CTC ATG AAC ATC ACT GGA GGA ACA AAC CTC AGC CTA
      Q   G   V   L   M   N   I   T   G   G   T   N   L   S   L

2755 TAT GAG GTT CAG GAA GCA GCA GAC ATT GTC GCT TCG GCG TCT GAT
      Y   E   V   Q   E   A   A   D   I   V   A   S   A   S   D

2800 CAA GAC GTA AAC ATG ATT TTC GGT TCT GTT ATT AAT GAA AAT CTA
      Q   D   V   N   M   I   F   G   S   V   I   N   E   N   L

2845 AAA GAT GAG ATT GTG GTG ACA GTG ATT GCA ACC GGC TTT ATC GAA
      K   D   E   I   V   V   T   V   I   A   T   G   F   I   E
                                                 HindIII
2890 CAA GAG AAG GAC GTG ACG AAG CCT CAG CGC CCA AGC TTA AAT CAA
      Q   E   K   D   V   T   K   P   Q   R   P   S   L   N   Q

2935 AGC ATC AAA ACA CAC AAT CAA AGT GTT CCG AAG CGT GAC GCA AAA
      S   I   K   T   H   N   Q   S   V   P   K   R   D   A   K

2980 CGT GAG GAA CCT CAG CAG CAG AAC ACA GTA AGC CGT CAT ACT TCA
      R   E   E   P   Q   Q   Q   N   T   V   S   R   H   T   S

3025 CAG CCG GCT GAT GAT ACG CTT GAC ATC CCG ACA TTC TTA AGA AAC
      Q   P   A   D   D   T   L   D   I   P   T   F   L   R   N

3070 CGT AAT AAA CGC GGC TAA TGTAAAGGACAAAATCGTTTTCGATTTTGTCTTTT
      R   N   K   R   G   -

3124 TTGTTTTTCTCTTCACACTTCCTTCTTATAAAGTCTTTTTCCCTATTGCTTTCCTTCGC

3183 TTAGTAACAAAACAGATAATTAGACCCATTTATTTTTGTGACATTTTTATCATTTTCAT

3242 ATATATGGAAATTGAATGACATGAAACGACAATATCTGTAATTCAGATTGTCTACAGTT

3301 AATATACAGCGATGTTCTGACAAACCATTCATTATTAAAAGGAGGGACGACACTTTTTT

3360 TAAAAAGCATGTTGAAAAAGGGGGATGAAA   ATG AGG AAA AAA ACG AAA AAC
                                       M   R   K   K   T   K   N

3410 AGA CTC ATC AGC TCT GTT TTA AGT ACA GTT GTC ATC AGT TCA CTG
      R   L   I   S   S   V   L   S   T   V   V   I   S   S   L
```

```
3455  CTG TTT CCG GGA GCA GCC GGG GCA AGC AGT AAA GTC ACC TCA CCT
       L   F   P   G   A   A   G   A   S   S   K   V   T   S   P

3500  TCT GTT AAA AAG GAG CTT CAA TCT GCG GAA TCC ATT CAA AAC AAG
       S   V   K   K   E   L   Q   S   A   E   S   I   Q   N   K
                                 HindIII
3545  ATT TCG AGT TCA TTA AAG AAA AGC TTT AAA AAG AAA GAA AAA ACG
       I   S   S   S   L   K   K   S   F   K   K   K   E   K   T

3590  ACT TTT CTG ATT AAA TTT AAA GAT CTG GCT AAC CCA GAA AAA GCG
       T   F   L   I   K   F   K   D   L   A   N   P   E   K   A

3635  GCA AAA GCG GCT GTT AAA AAA GCG AAA TCG AAG AAG CTG TCT GCC
       A   K   A   A   V   K   K   A   K   S   K   K   L   S   A

3680  GCT AAG ACG GAA TAT CAA AAG CGT TCT GCT GTT GTG TCA TCT TTA
       A   K   T   E   Y   Q   K   R   S   A   V   V   S   S   L

3725  AAA GTC ACA GCC GAT GAA TCC CAG CAA GAT GTC CTA AAA TAC TTG
       K   V   T   A   D   E   S   Q   Q   D   V   L   K   Y   L

3770  AAC ACC CAG AAA GAT AAA GGA AAT GCA GAC CAA ATT CAT TCT TAT
       N   T   Q   K   D   K   G   N   A   D   Q   I   H   S   Y

3815  TAT GTG GTG AAC GGG ATT GCT GTT CAT GCC TCA AAA GAG GTT ATG
       Y   V   V   N   G   I   A   V   H   A   S   K   E   V   M

3860  GAA AAA GTG GTG CAG TTT CCC GAA GTG GAA AAG GTG CTT CCT AAT
       E   K   V   V   Q   F   P   E   V   E   K   V   L   P   N

3905  GAG AAA CGG CAG CTT TTT AAG TCA TCC TCC CCA TTT AAT ATG AAA
       E   K   R   Q   L   F   K   S   S   S   P   F   N   M   K

3950  AAA GCA CAG AAA GCT ATT AAA GCA ACT GAC GGT GTG GAA TGG AAT
       K   A   Q   K   A   I   K   A   T   D   G   V   E   W   N
                                     HindIII
3995  GTA GAC CAA ATC GAT GCC CCA AAA GCT T
       V   D   Q   I   D   A   P   K   A
```

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the *ftsA-ftsZ* region of *B. subtilis*. The sequence extends from a *Pvu*II site through three *Hin*dIII sites. The ribosome-binding sites preceding each of the open reading frames are underlined. A promoterlike sequence for σ^A just upstream of the $ftsA_{Bs}$ gene is indicated, as is a promoterlike sequence within the $ftsA_{Bs}$ gene (1436 to 1462). The positions of various restriction sites are indicated.

Different derivatives of pBZ1 in which the $ftsZ_{Bs}$ gene was altered could be cloned directly into *E. coli*, which is consistent with the hypothesis that expression of $ftsZ_{Bs}$ was lethal to *E. coli*. Deletion of a 402-bp *Asu*II fragment resulted in an in-frame deletion near the 5′ end of the $ftsZ_{Bs}$ gene. The resultant plasmid could readily transform *E. coli*, although the transfected cells grew more slowly than control cells. Western analysis confirmed the presence of a truncated form of $FtsZ_{Bs}$ (Fig. 7B, lane 3). Additional plasmid derivatives were constructed that completely inactivated expression of $ftsZ_{Bs}$, and these plasmids had no effect on the growth of *E. coli*. These derivatives included one in which the *Asu*II fragment was inverted (resulting in the introduction of a nonsense mutation) and another in which the *Asu*II fragment was replaced with a Kan^r determinant.

A *Pst*I fragment containing the *ftsZ*, *ftsA*, and *ftsQ* genes of *E. coli* was cloned into the shuttle vector pMK4 by selecting for chloramphenicol resistance and complementation of the *ftsZ84*(Ts) mutant. When this plasmid was used to transfect *B. subtilis*, no transfectants were obtained, suggesting that the *E. coli* genes are lethal to *B. subtilis*. Thus, it appeared that the *fts* genes of these two organisms are not functionally interchangeable and are probably lethal in the other species.

Expression of $ftsZ_{Bs}$ is lethal to *E. coli*. From the results obtained so far, we assumed that overexpression of $ftsZ_{Bs}$ is lethal to *E. coli*. In addition, since the *Eco*RV-*Bgl*II fragment containing the $ftsZ_{Bs}$ gene could not be cloned in a plasmid even in the absence of external promoters, we assumed that this fragment must contain a promoter. The fragment contained 600 bp of DNA upstream of the $ftsZ_{Bs}$ gene which encoded the 3′ end of the $ftsA_{Bs}$ gene. It is known that in *E. coli* the strongest *ftsZ* promoters are located within the *ftsA* coding sequence (36). To remove any possible promoters, deletions were constructed starting from the *Sph*I site 600 bp

```
Bs    1'    MNNNELYVSLDLGTSNTKVIVGEMTGDSL-NIIGVGNVPSEGLKKGSIVDIDETVHS
             ...: :.:..::... ..:::.  :.. ::::::. :: :..::.. :....:..
Ec    1"  MIKATDRKLVVGLEIGTAKVAALVGEVLPDGMVNIIGVGSCPSRGMDKGGVNDLESVVKC

     57'  IRKAFDQAERMVGFPLRKAIVGVNGNYINIQDTNGVVAVSSENKEIQVEDVRRVMAAAQV
          ...:.:::: :.. ..... ....:..:. :.. :.:..   ...:.  ::: .:. .:.
     61"  VQRAIDQAELMADCQISSVYLALSGKHISCQNEIGMVPI--SEEEVTQEDVENVVHTAKS

    117'  VSVPHEQLIVDVIPKQFIVDGRDEITDPKKMLGVRLEVEGTLITGSKTILHNLLRCVERA
          :.:..:. ...:::... .: ...:..:  . :::....  :::   ... .:... :::
    119"  VRVRDEHRVLHVIPQEYAIDYQEGIKNPVGLSGVRMQAKVHLITCHNDMAKNIVKAVERC

    177'  GIEITDICLQPLAAGSAALSKDEKNLGVALIDIGGGSTTIAVFQNGHLTSTRVIPLGGEN
          :...... .. ::.. ..:..::..::: ..:::::. .:::. .: :  :.::: .:.
    179"  GLKVDQLIFAGLASSYSVLTEDERELGVCVVDIGGGTMDIAVYTGGALRHTKVIPYAGNV

    237'  ITKDISIGLRTSTEEAERVKKQLGHAYYDEASEDEIFEVTVIGTNQKQTFTQQEAANIIE
          .:.::. .. :....:: .: . : :  . ...::  ::. .:... ... .:. :..::
    239"  VTSDIAYAFGTPPSDAEAIKVRHGCALGSIVGKDESVEVPSVGGRPPRSLQRQTLAEVIE

    297'  ARVEEILEIVSEE-------LRSMGIT-DLPGGFVLTGGQAAMPGVMSLAQDVLQNNVRV
          .: .:.:.:.::        ::  :..  .:..:.:::::.:.. :. . :: :....::.
    299"  PRYTELLNLVNEEILQLQEKLRQQGVKHHLAAGIVLTGGAAQIEGLAACAQRVFHTQVRI

    349'  QGPNYIGVR-----DPQYMTGVGLIQFACRNARIQGRKIGFKMPEEAIQEIAVSSSEEQE
           .:  :.      .: : :.:::....
    359"  GAPLNITGLTDYAQEPYYSTAVGLLHYGKESHLNGEAEVEKRVTASVGSWIKRLNSWLRK

    404'  QHHHQNEVQQRPKGKQKTQAEHNKQSKMKKLLSMFWE

    419"  EF
```

FIG. 3. Comparison of the FtsA proteins of *B. subtilis* (Bs) and *E. coli* (Ec). The two FtsA proteins were compared by using the FASTP algorithm of Lipman and Person (15). Identical residues are indicated by a colon and conservative substitutions are shown by a dot. Homologies indicated after residue 310 were not picked up by the program and were added after inspection of the sequence. The deduced amino acid sequence for FtsA from *E. coli* was taken from Yi et al. (36).

upstream of the $ftsZ_{Bs}$ gene in pBZ1 by using *Bal*31 exonuclease and ligating in a *Bam*HI linker (Fig. 6). Since the parent plasmid cannot transform *E. coli*, any transformants that grew should no longer express $ftsZ_{Bs}$. In one of the derivatives that was obtained (pBZ1-1), most of the $ftsA_{Bs}$ coding sequence was deleted, but we estimated that the structural gene for $ftsZ_{Bs}$ and its ribosome-binding site should still be intact. Cells containing this plasmid produced $FtsZ_{Bs}$ that was barely detectable by Western analysis (data not shown).

The $ftsZ_{Bs}$ gene was cloned from pBZ1-1 into a shuttle vector, pMK4, under control of the *lac* regulatory system (pMKZ1-1, Fig. 6). The resultant plasmid was first isolated in *B. subtilis*. Purified plasmid DNA was prepared and used

```
Bs    1'  MLEFETNIDGLASIKVIGVGGGGNNAVNRMIENEVQGVEYIAVNTDAQALNLSKAEVKMQ
                       : :::::::::.:::..:. .  ..:::..::::::::. . .. ..:
Ec    1"   MFEPMELTNDAVIKVIGVGGGGGNAVEHMVRERIEGVEFFAVNTDAQALRKTAVGQTIQ

     61'  IGAKLTRGLGAGANPEVGKKAAEESKEQIEEALKGADMVFVTAGMGGGTGTGAAPVIAQI
          ::. .:.:::::::::::::.::.:..... .::.::::::.:::::::::::::::.:..
     60"  IGSGITKGLGAGANPEVGRNAADEDRDALRAALEGADMVFIAAGMGGGTGTGAAPVVAEV

    121'  AKDLGALTVGVVTRPFTFEGRKRQLQAAGGISAMKEAVDTLIVIPNDRILEIVDKNTPML
          ::::: :::.:::.::.:::.::   :. ::..... ::.::.::::..:.........:
    120"  AKDLGILTVAVVTKPFNFEGKKRMAFAEQGITELSKHVDSLITIPNDKLLKVLGRGISLL

    181'  EAFREADNVLRQGVQGISDLIATPGLINLDFADVKTIMSNKGSALMGIGIATGENRAAEA
          .:: .:..::. .::::..::. :::.:.::::::.:.::..: :.:: :.:.::.::.::
    180"  DAFGAANDVLKGAVQGIAELITRPGLMNVDFADVRTVMSEMGYAMMGSGVASGEDRAEEA

    241'  AKKAISSPLLEA-AIDGAQGVLMNITGGTNLSLYEVQEAADIVASASDQDVNMIFGSVIN
          :..::::::::. ...::.:::.:::.: .:.: : ....... . .........:. ..
    240"  AEMAISSPLLEDIDLSGARGVLVNITAGFDLRLDEFETVGNTIRAFASDNATVVIGTSLD

    300'  ENLKDEIVVTVIATGF-IEQEKDVTKPQRPSLNQSIKTHNQSVPKRDAKREEPQQQNTVS
            ...::. :::.:::. ...  ..:   .  ..:.... :   .   ..:. . ...:.
    300"  PDMNDELRVTVVATGIGMDKRPEITLVTNKQVQQPVMDRYQQHGMAPLTQEQKPVAKVVN

    359'  RHTSQPA--DDTLDIPTFLRNRNKRG
            ...:.:   : :::::.:::..
    360"  DNAPQTAKEPDYLDIPAFLRKQAD
```

FIG. 4. Comparison of the FtsZ proteins of *B. subtilis* (Bs) and *E. coli* (Ec). The two FtsZ proteins were compared as described in the legend to Fig. 3. The deduced amino acid sequence for *E. coli* FtsZ was taken from Yi and Lutkenhaus (35). Resequencing of the *E. coli ftsZ* gene revealed four nucleotide changes, two of which result in amino acid changes which are included in the FtsZ sequence.

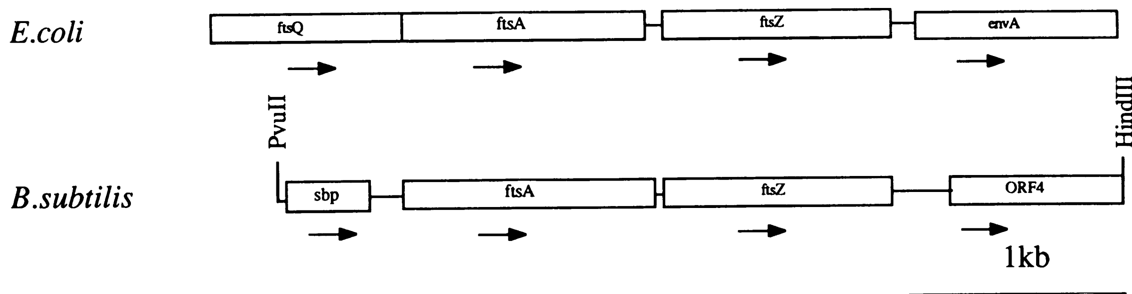


FIG. 5. Comparisons of gene organization in the *ftsZ* region of the *E. coli* and *B. subtilis* chromosomes. Arrows indicate gene orientation.

to transfect *E. coli* JM101 containing F′ *lacI*^{q}. JM101(pMKZ1-1) displayed an IPTG^s phenotype on plates and did not form colonies at an IPTG concentration above 0.05 mM. Examination of cells several hours after addition of IPTG revealed they were very filamentous. To determine the amount of FtsZ$_{Bs}$ that was required to inhibit cell division, Western analysis and cell number determinations were performed on cultures induced with various concentrations of IPTG. Analysis of these results indicated that when the FtsZ$_{Bs}$ level approached the level of the resident *E. coli* FtsZ, cell division was inhibited (data not shown).

It is possible that the FtsZ$_{Bs}$ was lethal to *E. coli* by inhibiting resident FtsZ function. In this way FtsZ$_{Bs}$ would resemble LacZ-FtsZ fusions, which are inhibitory to FtsZ function and cell division (33). Presumably these proteins, which lack FtsZ function, retain the ability to interact with FtsZ and inhibit its function. To test this possibility, we used the plasmid pBS58, which contains the *E. coli ftsQ*, *ftsA*, and *ftsZ* genes and leads to an elevated level of FtsZ. The plasmid pMKZ1-1 was introduced into JM101 containing the compatible plasmid pBS58, and the resultant strain was tested for the IPTG^s phenotype. The presence of pBS58 completely suppressed the IPTG^s phenotype, indicating that increasing the level of *E. coli* FtsZ could suppress the lethality of FtsZ$_{Bs}$.

Mapping of the *ftsZ*$_{Bs}$ gene. To map the *ftsZ*$_{Bs}$ gene, the 1.0-kbp *Hin*dIII fragment located just downstream of *ftsZ*$_{Bs}$ was cloned into pJH101 to give pJH101B. This plasmid cannot replicate in *B. subtilis* but can express chloramphenicol resistance in both *E. coli* and *B. subtilis*. Selecting for chloramphenicol resistance in *B. subtilis* results in homologous insertion of the plasmid, and then the Cm^r determinant can be readily mapped. Initial transduction with PBS1 showed weak linkage to *metC* (Table 2 and Fig. 8). Subsequent mapping showed that the Cm^r determinant was more tightly linked to *pyr* at 135 min. Several mutations that affect cell division, including *divI* (30), *ts31* (20), *ts12* (*tms12*), and *ts1* (2, 21), map in this region. It will be of interest to determine the relationship between *ftsZ*$_{Bs}$ and *ftsA*$_{Bs}$ and those genes indicated by these mutations.

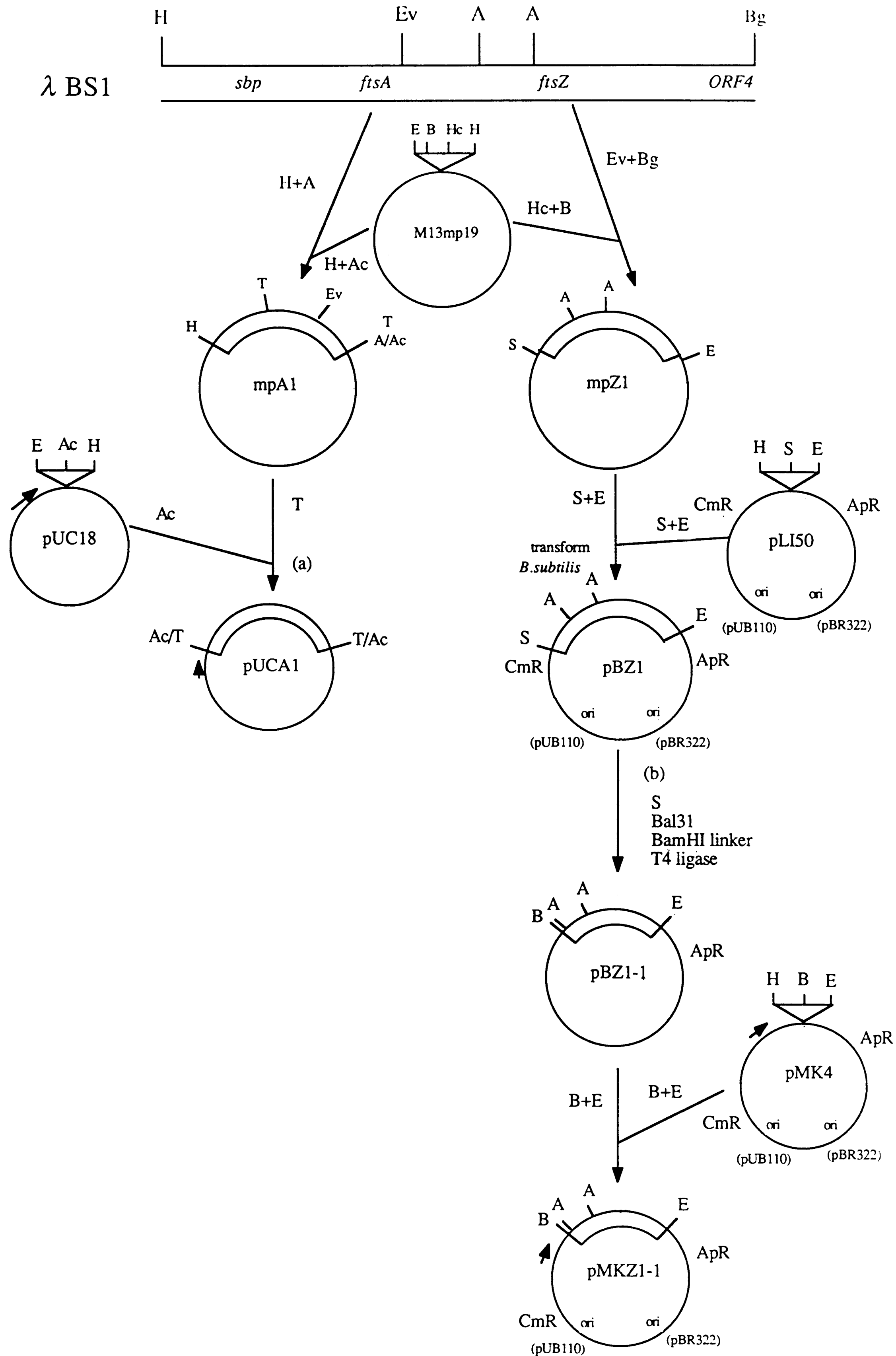
DISCUSSION

Genes showing strong homology to the *E. coli* cell division genes *ftsZ* and *ftsA* from *B. subtilis* have been cloned and sequenced. Since the genes encode proteins that are fairly well conserved, the genes appear to carry out the same functions in the two different organisms. Therefore, we have tentatively designated these genes from *B. subtilis ftsZ* and *ftsA*.

The two FtsZ proteins show a remarkable degree of amino acid identity, especially in the amino-terminal two-thirds of the protein. The 60% identity in this portion of the protein falls off to only 24% in the carboxy-terminal one-third of the protein to give an overall average of 50%. This degree of homology is comparable to that observed for DnaA, ribosomal protein L34, and the A subunit of DNA gyrase between these two organisms (22, 29). Thus, it places FtsZ among the more highly conserved group of proteins observed between these two organisms.

In *E. coli* the *ftsZ* gene is thought to be essential for cell division due to the phenotype of the *ftsZ84* temperature-sensitive mutation. Cells harboring this mutation display a filamentous phenotype at the nonpermissive temperature, even though DNA replication and segregation appear to be normal (18, 26). This led to the conclusion that a postsegregation step in cell division is impaired. Other experimental findings support a critical role for FtsZ in cell division. Overproduction of FtsZ (three- to sevenfold) results in stimulation of cell division in the form of the minicell phenotype, which implicates FtsZ as being rate limiting for cell division (34). In *E. coli* the presence of *ftsZ* on a multicopy plasmid is sufficient to lead to overproduction of FtsZ and the minicell phenotype. Introduction of pBZ1 carrying the *ftsZ*$_{Bs}$ gene into *B. subtilis* did not result in overproduction of FtsZ or the minicell phenotype. We were unable to make constructs that included the promoter upstream of *ftsA*$_{Bs}$, which would presumably increase the level of FtsZ, to determine whether this would induce the minicell phenotype.

FIG. 6. Subcloning the *ftsZ*$_{Bs}$ and *ftsA*$_{Bs}$ genes into plasmid vectors. (A) Cloning the *ftsA*$_{Bs}$ gene. Initially a *Hin*dIII-*Asu*II fragment containing the *ftsA*$_{Bs}$ gene was cloned from λBS1 into M13mp19. Subsequently the *ftsA*$_{Bs}$ structural gene without its promoter was subcloned on a *Taq*I fragment into pUC18 in the proper orientation to be expressed from the *lac* promoter. The correct orientation was obtained by screening for an IPTG^s phenotype. (B) Cloning the *ftsZ*$_{Bs}$ gene. Initially the *ftsZ*$_{Bs}$ gene on an *Eco*RV-*Bgl*II fragment was subcloned from λBS1 into M13mp19. Next, the *ftsZ*$_{Bs}$ gene was placed into a shuttle vector by cloning an *Sph*I-*Eco*RV fragment into pL150. The resultant plasmid, pBZ1, was obtained in *B. subtilis* but could not be introduced into *E. coli*. The region upstream of *ftsZ*$_{Bs}$ was removed with *Bal*31 to give pBZ1-1, which could be introduced into *E. coli*. Finally, the *ftsZ*$_{Bs}$ gene was placed under *lac* control in the shuttle vector pMK4 to give pMKZ1-1. Arrows indicate the position and orientation of the *lac* promoter. Restriction sites: *Acc*I, Ac; *Asu*II, A; *Bam*HI, B; *Bgl*II, Bg; *Eco*RI, E; *Eco*RV, Ev; *Hin*cII, Hc; *Hin*dIII, H; *Sph*I, S; *Taq*I, T.


λ BS1
H
Ev
A
A
Bg
sbp
ftsA
ftsZ
ORF4
H+A
Ev+Bg
E B Hc H
M13mp19
Hc+B
H+Ac
mpA1
mpZ1
T
Ev
A/Ac
S
E
pUC18
Ac
(a)
pUCA1
Ac/T
T/Ac
S+E
transform
B.subtilis
H S E
CmR
ApR
pLI50
ori
(pUB110)
(pBR322)
pBZ1
(b)
Bal31
BamHI linker
T4 ligase
pBZ1-1
B
H B E
pMK4
B+E
pMKZ1-1

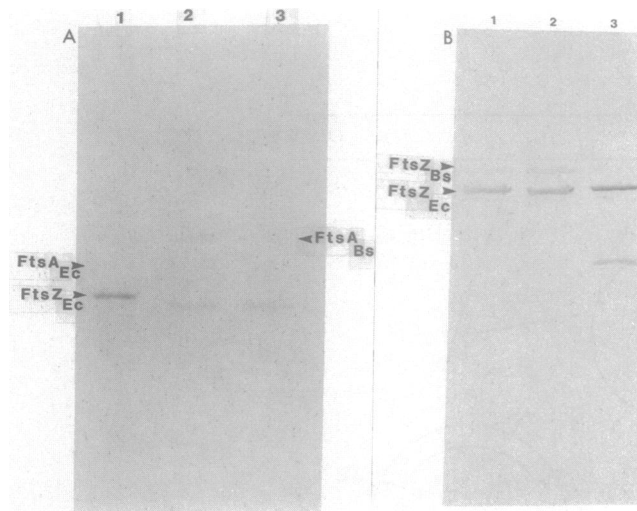


FIG. 7. Detection of the $ftsZ_{Bs}$ and $ftsA_{Bs}$ gene products. (A) Autoradiogram of [^{35}S]methionine-labeled maxicells analyzed by SDS-PAGE. JFL101 (F'::Tn*9 lacI*^{q}) containing pZAQ (*E. coli ftsA* and *ftsZ* genes), lane 1; infected with mpA1 ($ftsA_{Bs}$), lane 2; and infected with M13mp19, lane 3. (B) Western analysis of *B. subtilis* FtsZ protein expressed in *E. coli*. JFL101 (F'::Tn*9 lacI*^{q}) infected with mpZ1 ($ftsZ_{Bs}$), lane 1; mpZ1 ($ftsZ_{Bs}$) plus 0.3 mM IPTG, lane 2; and transfected with pBZ1Δ*Asu*II, in which a 402-bp *Asu*II fragment deletion results in an in-frame deletion near the 5' end of the gene, lane 3. $FtsZ_{Ec}$, *E. coli* FtsZ.

In *E. coli*, FtsZ is the target of the cell division inhibitor SulA, which is produced as part of the SOS response following DNA damage (13, 17). An inducible SOS-like system has been found in *B. subtilis*, which includes a filamentation response (16). It is possible that *B. subtilis* also has a homolog of the *sulA* gene and that inhibition of cell division occurs by a similar mechanism. Recently, we characterized a number of *sulB* mutations, which map in *ftsZ* and make it resistant to SulA, and noted that these mutations occur in regions that are conserved between the two genes (Bi and Lutkenhaus, manuscript in preparation).

The $ftsZ_{Bs}$ gene does not complement the *ftsZ84* temperature-sensitive mutation, and in fact, its expression is lethal to *E. coli*. We conclude that this is due to the inhibition of *E. coli* FtsZ function, since expression of *B. subtilis ftsZ* results in filamentation and this can be overcome by increasing the level of *E. coli* FtsZ. Expression of truncated or missense forms of *E. coli* FtsZ result in inhibition of cell division and cell death by inhibiting wild-type FtsZ, since the inhibition can be overcome by increased wild-type FtsZ (33). Previously, we proposed that FtsZ may be active as an oligomer and that the formation of mixed oligomers between wild-type FtsZ and nonfunctional forms of FtsZ, such as LacZ-FtsZ fusions, may be lethal. It may be that $FtsZ_{Bs}$ forms a mixed oligomer with *E. coli* FtsZ which is nonfunctional.

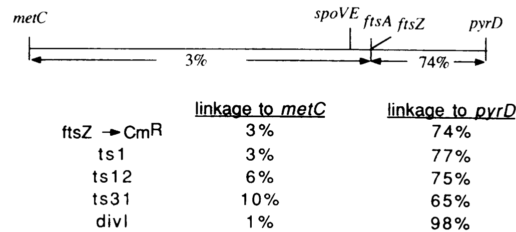


FIG. 8. Chromosomal map location of the $ftsZ_{Bs}$ region. The location of $ftsZ_{Bs}$ was determined by PBS1 transduction of Cm^r inserted just downstream of $ftsZ_{Bs}$. The numbers below the line indicate the cotransduction of the two markers as shown in Table 2. The linkages of known *B. subtilis* cell division mutations are indicated (taken from references 2 and 20).

Although we have not examined it in detail, the $ftsA_{Bs}$ gene did not complement an *E. coli* temperature-sensitive *ftsA* mutant, and expression of $ftsA_{Bs}$ resulted in filamentation. In this respect it is similar to the $ftsZ_{Bs}$ complementation and to effects observed with other *B. subtilis* genes when they are introduced into *E. coli*. For example, $dnaA_{Bs}$ (1) and $polC_{Bs}$ (23) cannot complement mutations in their corresponding *E. coli* homologs. Thus, the lack of complementation between these genera may be rather common, at least for genes involved in DNA replication and cell division.

The high degree of amino acid identity between the two FtsZ proteins made possible the detection of $FtsZ_{Bs}$ with antiserum prepared against the purified *E. coli* FtsZ protein. This was important in the cloning, since the DNA sequences are only 50% homologous, making it impossible to detect the gene by Southern hybridization (5). An additional feature of the cloning method should be noted. Since the *ftsZ* gene of *E. coli* is essential, it could not be deleted, and all plaques from the library showed up as faintly positive due to FtsZ from lysed cells; however, the clones containing the $ftsZ_{Bs}$ gene showed up as stronger positives, allowing their detection.

In *E. coli* the *ftsZ* gene is preceded by the *ftsA* gene, and both lie within a large cluster of genes involved with cell wall physiology (7, 18). In *B. subtilis* the $ftsZ_{Bs}$ gene is also preceded by the $ftsA_{Bs}$ gene, but the flanking genes do not show any homology to those present in *E. coli*. A search of the protein sequence data base of the Protein Identification Resource (Release 16) revealed no significant homologies to the *B. subtilis* flanking genes. It is surprising that these two *fts* genes remained linked, whereas *ftsQ*, which overlaps the *ftsA* gene in *E. coli*, is not immediately linked. Perhaps there is a functional significance for the juxtapositioning of the *ftsA* and *ftsZ* genes. In any event, the large cluster of genes related to cell wall physiology found in *E. coli* is not retained intact in *B. subtilis*. However, the determination that a *B. subtilis* cell division mutation maps in this region but just upstream of $ftsA_{Bs}$ (see below) would indicate that this cell division gene cluster is more extensive.

TABLE 2. Chromosomal mapping of the *B. subtilis ftsA-ftsZ* region by PBS1 transduction

Donor[a]	Recipient	Selected/unselected markers	No. of recombinants tested	No. (%) of recombinants lacking selected marker
ftsZ→Cm^r	*metC*::Tn*917*	Cm^r/Ery^r	150	5 (3)
ftsZ→Cm^r	*pyr-83*::Tn*917*	Cm^r/Ery^r	82	58 (71)
pyr-82::Tn*917*	*ftsZ*→Cm^r	Ery^r/Cm^r	272	193 (71)
pyr-83::Tn*917*	*ftsZ*→Cm^r	Ery^r/Cm^r	100	83 (83)

[a] *ftsZ*→Cm^r refers to a Cm^r gene inserted just downstream of *ftsZ* on the *B. subtilis* chromosome.

The region on the *B. subtilis* genetic map where the $ftsZ_{Bs}$ and $ftsA_{Bs}$ genes are located includes several genes associated with cell division and several sporulation genes. Recently, we determined that the *tms-12* mutation (4) can be rescued by λBS1, but that the gene corresponding to this mutation maps upstream of $ftsA_{Bs}$ (on a 1.8-kb *Bam*HI-*Hin*dIII fragment). Other genetic markers that relate to cell division and map in this region, *divI* (30), *ts31* (20), *tsl* (2), and *tsl2* (thought to be in the same gene as *tms-12* [2]), we have not tested. After this work was completed, E. J. Harry and R. G. Wake (personal communication) informed us that they had mapped the *tms-12* mutation to the same location upstream of $ftsA_{Bs}$. In addition, they determined that the *tsl* mutation could be rescued by a restriction fragment that, according to our sequence data, is internal to the $ftsZ_{Bs}$ gene. These data confirm a functional conservation of the *ftsZ* gene.

Recently, it was pointed out to us by T. Leighton that a phage carrying the same insert as λBS1 (but in the opposite orientation) had previously been isolated by Piggot et al. (24) in cloning the *spoVE* gene. The *spoVE* gene is located on the 2.8-kb *Eco*RI fragment (Fig. 1) approximately 5 kb upstream of *ftsA*. In addition, they showed that the *spoIIG55*, *spoVD156*, and *spoIIIE24* markers could not be rescued by this phage.

In examining the DNA sequence, we observed a good consensus promoterlike sequence for the major vegetative sigma just upstream of the $ftsA_{Bs}$ gene. It matches the canonical sequence in spacing as well as in the most conserved positions. This would imply that these genes are expressed during vegetative growth. Transcripts initiating at this promoter would presumably continue through the $ftsZ_{Bs}$ gene, since it follows $ftsA_{Bs}$ so closely and no transcription terminators are apparent between them. However, this does not appear to be the only promoter from which the $ftsZ_{Bs}$ gene is expressed. Our studies on cloning the $ftsZ_{Bs}$ gene in *E. coli* suggest that at least one additional promoter is located within the 600-bp 3′ end of the $ftsA_{Bs}$ gene that is functional in *E. coli*. This promoter may also function in *B. subtilis*. A possible candidate occurs at positions 1436 to 1464 (Fig. 2). This promoter candidate differs in only one position (in the −35 and −10 regions) from the *lacUV5* promoter, which is a good promoter in *E. coli* but weak in *B. subtilis* (12).

The *ftsA* gene is thought to be an essential cell division gene in *E. coli*. Temperature-sensitive mutations that map in this gene confer a characteristic filamentous morphology at the nonpermissive temperature, with indentations along the filament. This has led to the idea that cell division is blocked at a later step than in *ftsZ* mutants (7). Recently it was suggested that a small segment of FtsA (residues 305 to 357 in the *E. coli* sequence, Fig. 3) shows homology to the cell cycle proteins Cdc-2 and Cdc-28 of *Saccharomyces cerevisiae* (Robinson et al., letter, Nature **328**:766, 1987). Comparison of this region with the corresponding segment of the *B. subtilis* FtsA protein reveals several significant differences. The *B. subtilis* FtsA protein is missing seven amino acids in this region, and several other amino acids identical between *E. coli* and yeast cells are not conserved in *B. subtilis*. In addition, the potential nucleotide-binding site and phosphorylation site observed in the *E. coli* FtsA protein, which indicate that it may be a kinase, are not present in the *B. subtilis* FtsA protein. Therefore, we believe that the observed homology with the yeast sequence is not functionally significant.

Recently, it was reported that the temperature-sensitive stage II mutation, *spo-279* (37), blocking formation of the asymmetric septum, maps in a new sporulation gene designated *spoIIN*. Cloning and marker rescue experiments indicate that this mutation maps in or near the $ftsA_{Bs}$ gene (T. Leighton et al., FASEB J. 2:A1017, abstr. no. 4188, 1988). Examination of the *spo-279.2* strain during vegetative growth revealed the additional phenotype of extreme filamentation at high temperature, although colony-forming ability was not affected (unpublished observation). This indicates that the *spo-279* mutation affects vegetative septation, although it is somewhat leaky, allowing colony formation. It will be intriguing to discover whether there is any relationship between *spoIIN* and $ftsA_{Bs}$.

In summary, sequence data confirmed that *B. subtilis* contains homologs of the *E. coli* cell division genes *ftsZ* and *ftsA*. Expression of the *B. subtilis* homologs in *E. coli* results in filamentation and cell death. Available information about the location of known *B. subtilis* cell division mutations indicates that the function of the *ftsZ* gene is also conserved.

ACKNOWLEDGMENTS

We are grateful to T. Leighton, E. J. Harry, and R. G. Wake for communicating results before publication and to George Stewart, Christine Buchannan, Ivan Goldberg, and Charles P. Moran, Jr., for sending strains.

This work was supported by Public Health Service grant GM29764 from the National Institutes of Health and by a Biomedical Research Support grant.

LITERATURE CITED

1. **Andrup, L., T. Atlung, N. Ogasawara, H. Yoshikawa, and F. G. Hansen.** 1988. Interaction of the *Bacillus subtilis* DnaA-like protein with the *Escherichia coli* DnaA protein. J. Bacteriol. **170**:1333–1338.
2. **Callister, H. J., and R. G. Wake.** 1981. Characterization and mapping of temperature-sensitive division initiation mutations in *Bacillus subtilis*. J. Bacteriol. **145**:1042–1051.
3. **Churchward, G., D. Belin, and Y. Nagamine.** 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene **31**:165–171.
4. **Copeland, J. C., and J. Marmur.** 1968. Identification of conserved genetic functions in *Bacillus* by use of temperature-sensitive mutants. Bacteriol. Rev. **32**:302–312.
5. **Corton, J. C., J. E. Ward, Jr., and J. Lutkenhaus.** 1987. Analysis of cell division gene *ftsZ* (*sulB*) from gram-negative and gram-positive bacteria. J. Bacteriol. **169**:1–7.
6. **Dedonder, R. A., J. A. Lepesant, J. Lepesant-Kejzlarova, A. Billault, M. Steinmitz, and F. Kunst.** 1977. Construction of a kit of reference strains for rapid genetic mapping in *Bacillus subtilis* 168. Appl. Environ. Microbiol. **33**:989–993.
7. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. Morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Dubnau, D., R. Davidoff-Abelson, B. Scher, and C. Cirigliano.** 1973. Fate of transforming deoxyribonucleic acid after uptake by competent *Bacillus subtilis*: phenotypic characterization of radiation-sensitive, recombination-deficient mutants. J. Bacteriol. **114**:273–286.
9. **Ferrari, E., D. J. Henner, and J. A. Hoch.** 1981. Isolation of *Bacillus subtilis* genes from a Charon 4A library. J. Bacteriol. **146**:430–432.
10. **Ferrari, E., A. Nguyen, D. Lang, and J. A. Hoch.** 1983. Construction and properties of an integrable plasmid for *Bacillus subtilis*. J. Bacteriol. **154**:1513–1515.
11. **Fitz-James, P. C., and I. E. Young.** 1969. Morphology of sporulation, p. 39. *In* G. W. Gould and A. Hurst (ed.), The bacterial spore. Academic Press, London.
12. **Henkin, T. M., and A. L. Sonenshein.** 1987. Mutations of the *Escherichia coli lacUV5* promoter resulting in increased expres-

sion in *Bacillus subtilis*. Mol. Gen. Genet. **209**:467–474.
13. **Huisman, O., and R. D'Ari.** 1981. An inducible DNA replication-cell division coupling mechanism in *E. coli*. Nature (London) **290**:797–799.
14. **Jones, C. A., and I. B. Holland.** 1985. Role of the SfiB (FtsZ) protein in division inhibition during the SOS response in *E. coli*: FtsZ stabilizes the inhibitor SfiA in maxicells. Proc. Natl. Acad. Sci. USA **82**:6045–6049.
15. **Lipman, D. J., and W. R. Person.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
16. **Love, P. E., and R. E. Yasbin.** 1984. Genetic characterization of the inducible SOS-like system of *Bacillus subtilis*. J. Bacteriol. **160**:910–920.
17. **Lutkenhaus, J. F.** 1983. Coupling of DNA replication and cell division: *sulB* is an allele of *ftsZ*. J. Bacteriol. **154**:1339–1346.
18. **Lutkenhaus, J. F., H. Wolf-Watz, and W. D. Donachie.** 1980. Organization of genes in the *ftsA-envA* region of the *Escherichia coli* genetic map and identification of a new *fts* locus (*ftsZ*). J. Bacteriol. **142**:615–620.
19. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–77.
20. **Miyakawa, Y., and T. Komano.** 1981. Study on the cell cycle of *Bacillus subtilis* using temperature-sensitive mutants. I. Isolation and genetic analysis of mutants defective in septum formation. Mol. Gen. Genet. **181**:207–214.
21. **Nukushina, J.-I., and Y. Ikeda.** 1969. Genetic analysis of the developmental processes during germination and outgrowth of *Bacillus subtilis* spores with temperature-sensitive mutants. Genetics **63**:63–74.
22. **Ogasawara, N., S. Moriya, K. von Meyenburg, F. G. Hansen, and H. Yoshikawa.** 1985. Conservation of genes and their organization in the chromosomal replication origin region of *Bacillus subtilis* and *Escherichia coli*. EMBO J. **4**:3345–3350.
23. **Ott, R. W., L. E. Goodman, and A. T. Ganesan.** 1987. Expression of the *Bacillus subtilis polC* gene in *Escherichia coli*. Mol. Gen. Genet. **207**:335–341.
24. **Piggot, P. J., K.-F. Chak, and U. K. Bugaichuk.** 1986. Isolation and characterization of a clone of the *spoVE* locus of *Bacillus subtilis*. J. Gen. Microbiol. **132**:1875–1881.
25. **Piggot, P. J., and J. A. Hoch.** 1985. Revised genetic linkage map of *Bacillus subtilis*. Microbiol. Rev. **49**:158–179.
26. **Ricard, M., and Y. Hirota.** 1973. Process of cellular division in *Escherichia coli*: physiological study on thermosensitive mutants defective in cell division. J. Bacteriol. **116**:314–322.
27. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-encoded proteins. J. Bacteriol. **137**: 692–693.
28. **Sullivan, M. A., R. E. Yasbin, and R. E. Young.** 1984. New shuttle vectors for *Bacillus subtilis* and *Escherichia coli* which allow rapid detection of inserted fragments. Gene **29**:21–26.
29. **Swanberg, S. L., and J. C. Wang.** 1987. Cloning and sequencing of the *Escherichia coli gyrA* gene coding for the A subunit of DNA gyrase. J. Mol. Biol. **197**:729–736.
30. **Van Alstyne, D., and M. I. Simon.** 1971. Division mutants of *Bacillus subtilis*: isolation and PBS1 transduction of division-specific markers. J. Bacteriol. **108**:1366–1379.
31. **Vandeyar, M. A., and S. A. Zahler.** 1986. Chromosomal insertions of Tn*917* in *Bacillus subtilis*. J. Bacteriol. **167**:530–534.
32. **Vieira, J., and J. Messing.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**:259–268.
33. **Ward, J. E., and J. F. Lutkenhaus.** 1984. A *lacZ-ftsZ* gene fusion is an analog of the cell division inhibitor *sulA*. J. Bacteriol. **157**:815–820.
34. **Ward, J. E., and J. F. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicells in *E. coli*. Cell **42**:941–949.
35. **Yi, Q.-M., and J. Lutkenhaus.** 1985. The nucleotide sequence of the essential cell division gene *ftsZ*. Gene **36**:241–247.
36. **Yi, Q.-M., S. Rockenbach, J. E. Ward, and J. Lutkenhaus.** 1985. Structure and expression of the cell division genes *ftsQ*, *ftsA*, and *ftsZ*. J. Mol. Biol. **184**:399–412.
37. **Young, M.** 1985. Genetic mapping of sporulation operons in *Bacillus subtilis* using a thermosensitive sporulation mutant. J. Bacteriol. **122**:1109–1116.