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Analysis of Cell Division Gene *ftsZ* (*sulB*) from Gram-Negative and Gram-Positive Bacteria

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The *ftsZ* (*sulB*) gene of *Escherichia coli* codes for a 40,000-dalton protein that carries out a key step in the cell division pathway. The presence of an *ftsZ* gene protein in other bacterial species was examined by a combination of Southern blot and Western blot analyses. Southern blot analysis of genomic restriction digests revealed that many bacteria, including species from six members of the family *Enterobacteriaceae* and from *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*, contained sequences which hybridized with an *E. coli ftsZ* probe. Genomic DNA from more distantly related bacteria, including *Bacillus subtilis*, *Branhamella catarrhalis*, *Micrococcus luteus*, and *Staphylococcus aureus*, did not hybridize under minimally stringent conditions. Western blot analysis, with anti-*E. coli* FtsZ antiserum, revealed that all bacterial species examined contained a major immunoreactive band. Several of the *Enterobacteriaceae* were transformed with a multicopy plasmid encoding the *E. coli ftsZ* gene. These transformed strains, *Shigella sonnei*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*, were shown to overproduce the FtsZ protein and to produce minicells. Analysis of [^{35}S]methionine-labeled minicells revealed that the plasmid-encoded gene products were the major labeled species. This demonstrated that the *E. coli ftsZ* gene could function in other bacterial species to induce minicells and that these minicells could be used to analyze plasmid-endoced gene products.

A number of genes essential for cell division in *Escherichia coli* have been mapped to the 2-min region of the genetic map. These genes have been identified by mapping conditionally lethal mutations which, at the nonpermissive temperature, block septum formation and give rise to multinucleated filaments (8). The *ftsZ* (*sulB*) gene and two neighboring cell division genes, *ftsQ* and *ftsA*, map in this region and have been shown to be tightly clustered and transcribed in the same direction and to form an atypical operon (20, 25, 29).

Experimental evidence suggests that the *ftsZ* gene is involved in an early, crucial step in the formation of the cell division septum. Analysis of double mutants that are temperature sensitive for cell elongation and septation has indicated that *ftsZ* acts earlier than *ftsQ*, *ftsA*, or *ftsI* (*pbpB*) in septation since formation of septumlike constrictions required only *ftsZ* (2). Studies on the effect of overproduction of the FtsZ protein have revealed that increasing the level of FtsZ causes an increase in the number of cell division events resulting in the production of minicells (26). Thus, the amount of FtsZ protein may control a rate-determining early step in the formation of the septum.

Substantial evidence indicates that the FtsZ protein is the target of the cell division inhibitor protein SulA, which is induced when DNA-damaging agents activate a series of cellular events termed the SOS response (for a review of SOS, see reference 11). SulA is stabilized in a *lon* mutant, enhancing its inhibitory effect and leading to lethal filamentation after SOS induction (18). *sulB* (*sfiB*) mutations that suppress this lethal effect of SulA have been isolated and found to map in the *ftsZ* gene (8, 14). It is possible that the *sulB* and *sfiB* mutations result in an altered FtsZ protein that is resistant to SulA but still able to carry out its cell division function. The overproduction of wild-type FtsZ is also able to override the SOS-induced lethal filamentation in *lon* mutants, supporting the hypothesis that FtsZ is the target of SulA (15). Additional evidence suggests that FtsZ and SulA interact directly since FtsZ increases the half-life of SulA in maxicells but the SulB form does not (9). This inhibition of FtsZ by SulA must be reversible since SOS-induced filaments can recover once SulA is removed, even in the absence of de novo protein synthesis (17). These results implicating FtsZ as the target of SulA suggest a key role for FtsZ in cell division control.

Little is known about the cell division machinery in other bacterial species. Very few genes which might play a role in cell division in different species have been characterized, and none of the *E. coli* cell division genes have been shown to have a direct counterpart in other bacteria. To learn more about cell division and the role of *ftsZ*, we analyzed other bacteria for the existence of an *ftsZ* gene and examined the relatedness in structure and function of *ftsZ* genes in other bacteria to the gene in *E. coli*. Our results showed that most if not all bacteria contain an *ftsZ* gene, since all bacteria examined in this sutdy contained genomic DNA sequences which hybridized to an *ftsZ* gene probe or contained a protein which was immunologically related to the purified FtsZ protein of *E. coli*, or both. Also, the *E. coli ftsZ* gene could function in other members of the family *Enterobacteriaceae*, since introduction of the *E. coli ftsZ* gene on a multicopy plasmid induced the minicell phenotype.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains used in this investigation are listed in Table 1. Plasmid pZAQ (Fig. 1) contains a 4.4-kilobase (kb) *Pst*I-*Cla*I fragment from λ16-25 subcloned into pBR322. Plasmid pKZAQ was constructed in this laboratory by Bharati Sanjanwala, by subcloning the

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TABLE 1. Bacterial species and strains in this study

Species (strain)	Source
Agrobacterium tumefaciens A348	E. Nester
Bacillus subtilus W168	I. Goldberg
Branhamella catarrhalis	R. Sobieski
Citrobacter diversus	R. Sobieski
Enterobacter aerogenes	R. Sobieski
Escherichia coli GC4689	R. D'Ari
Escherichia coli W3110	Laboratory collection
Escherichia coli LE392	Laboratory collection
Klebsiella pneumoniae	R. Hirschberg
Micrococcus luteus	R. Sobieski
Proteus mirabilis	M. Inouye
Pseudomonas aeruginosa	D. Furtado
Salmonella typhimurium LT2	K. Sanderson
Serratia marcescens	R. Sobieski
Shigella sonnei 11060	D. Furtado
Staphylococcus aureus	D. Furtado
Streptococcus faecalis	D. Furtado

~1.5-kb *Sal*I fragment from plasmid pUC4K (Pharmacia Fine Chemicals) containing the kanamycin resistance gene into the *Sal*I site in pZAQ.

Media and growth conditions. LB medium contained yeast extract (5 g/liter) (Difco Laboratories), Difco tryptone (10 g/liter), and NaCl (5 g/liter). This medium was solidified by the addition of Difco agar (15 g/liter) for plates. All cultures were grown at 37°C except *Micrococcus luteus* and *Agrobacterium tumefaciens*, which were grown at 30°C.

Southern hybridizations. For Southern hybridizations, chromosomal DNA isolated by the method of Harris-Warrick et al. (7) was digested with an excess of the indicated restriction enzyme (Bethesda Research Laboratories). Approximately equal amounts of the digested DNA were electrophoresed in 1.0% agarose gels. Chromosomal DNA digestions which contained *ftsZ* sequences on fragments larger than 13 kb were also analyzed on 0.5% agarose gels to obtain a better estimate of the fragment size. The gels were photographed and then prepared for capillary transfer to GeneScreen Plus transfer membranes (New England Nuclear Corp.) by the specifications of the manufacturer. From a digestion of pZAQ with *Eco*RI and *Bst*EII (Bethesda Research Laboratories), an internal 863 base-pair fragment from within the *ftsZ* gene (Fig. 1) was isolated from 0.7% agarose gels with the aid of DEAE-cellulose membrane strips (NA-45; Schleicher & Schuell, Inc.) which were used by the specifications of the manufacturer. The isolated fragment was extracted twice with phenol-chloroform, ethanol precipitated, and then labeled with [^{32}P]dATP or [^{32}P]dCTP (New England Nuclear Corp.) by nick-translation to a specific activity of ca. 10^8 cpm/μg. The transfer membranes were prehybridized, hybridized, and washed as directed by the manufacturer. The transfer membranes containing the genomic DNA of *Bacillus subtilis, Branhamella catarrhalis, M. luteus*, and *Staphylococcus aureus* were also washed after hybridization under less stringent conditions; the membranes were washed twice with 4× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 25°C for 30 min each time, followed by two washes of 30 min each with 4× SSC–1% sodium dodecyl sulfate (SDS) and two washes of 30 min each with 4× SSC at 25°C. The transfer membranes were wrapped in two sheets of plastic film, and autoradiography was performed for 8 to 72 h at −70°C with X-ray film and an intensifying screen.

Preparation of cell lysates. Cell lysates of gram-negative, rod-shaped bacteria were prepared from cultures grown to an optical density at 540 nm of 0.4 to 0.5. Samples (1 to 3 ml each) of the cell cultures were centrifuged and suspended in a small volume of SDS-sample buffer (62.5 mM Tris hydrochloride [pH 6.8], 1% SDS, 10% glycerol, 5% 2-mercaptoethanol). The lysates of the gram-positive species and *Branhamella catarrhalis* were prepared by growing approximately 100 ml of cell culture to an optical density at 540 nm of 0.4 to 0.5, pelleting the cells, washing in TE (10 mM Tris hydrochloride [pH 8.0] and 1 mM EDTA), pelleting again, suspending in 5 ml of TE, and sonicating for a total time of 4 min. A small amount of the sonicated extract was mixed with an equal volume of SDS-sample buffer. All samples were heated at 100°C for 10 min.

Immunoblotting procedures. Proteins electrophoresed on a 10% polyacrylamide-SDS gel were electrophoretically transferred to nitrocellulose overnight at 150 mA as described by Burnette (4). Proteins antigenically related to FtsZ were detected by an indirect immunostaining procedure with a rabbit polyclonal antisera raised against purified denatured FtsZ and goat antirabbit immunoglobulin G coupled to horseradish peroxidase. Staining of immunoreactive bands was performed in the presence of hydrogen peroxide and 4-chloro-1-naphthol.

Purification and labeling of minicells. Minicells were purified by the method of Reeve (19). Plasmid-encoded proteins were identified by incubating approximately 10^{10} minicells for 1 h in 0.6 ml of M9 minimal medium (0.4% glucose, 22 mM potassium phosphate monobasic, 42 mM sodium phosphate dibasic, 19 mM ammonium chloride, 1 mM magnesium sulfate) supplemented with 10% methionine assay medium (Difco Laboratories) and then labeling the samples for 1 h in the presence of 60μCi of [^{35}S]methionine (New England Nuclear Corp.). Minicells were pelleted and suspended in SDS-sample buffer, and labeled proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography.

RESULTS

***ftsZ* DNA sequence homology in gram-negative and gram-positive bacteria.** An *E. coli ftsZ* gene probe was hybridized to chromosomal DNA from 14 different bacterial species by using the technique of Southern (24). The *ftsZ* gene probe used was an internal *Eco*RI-*Bst*EII fragment (Fig. 1) purified from plasmid pZAQ. *Eco*RI digests of chromosomal DNAs

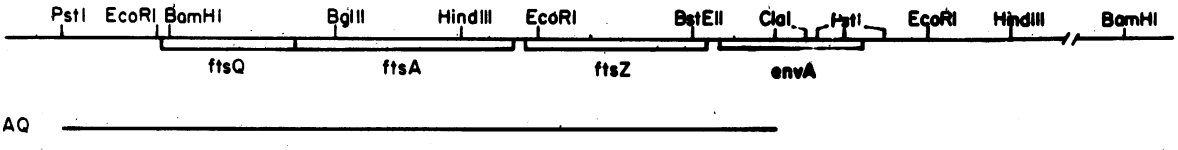

FIG. 1. Organization and restriction map of the genes in the 2-min region. The *Eco*RI-*Bst*EII fragment within the *ftsZ* gene was used as a probe in the Southern hybridization experiments. Plasmid pZAQ contains a *Pst*I-*Cla*I restriction fragment from the 2-min region cloned into plasmid pBR322.

from 11 different rod-shaped species of bacteria which were transferred to a nylon membrane and hybridized with the nick-translated *ftsZ* probe are shown in Fig. 2. When compared with the *E. coli* genomic digest (Fig. 2), *Citrobacter diversus*, *Shigella sonnei*, *Serratia marcescens*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* (lanes 2 through 7, respectively) were found to contain DNA sequences which had high degrees of homology with the *E. coli ftsZ* gene. Chromosomal DNA from *Proteus mirabilis* (lane 8) had less homology than that from the other species of *Enterobacteriaceae*. DNA from *Pseudomonas aeruginosa* (lane 9) and *A. tumefaciens* (lane 11) showed the least homology to the *ftsZ* gene of all the gram-negative, rod-shaped bacteria examined.

Genomic DNAs from a number of more distantly related rod- and coccal-shaped bacteria were tested for *ftsZ*-like sequences. The genomic DNA from *Bacillus subtilis* digested with *Eco*RI (Fig. 2, lane 10), *Pst*I, *Bam*HI, or *Hin*dIII (data not shown) did not possess enough sequence homology to hybridize to the *ftsZ* probe. The digested genomic DNA of *Branhamella catarrhalis*, a gram-negative coccal-shaped bacterium, and *Staphylococcus aureus* and *M. luteus*, two gram-positive coccal-shaped bacteria, did not hybridize under the standard stringency conditions (data not shown). When the hybridization was repeated for these bacterial species under conditions of low stringency, no hybridization was detected. This could mean either a great divergence from the *E. coli ftsZ* gene at the nucleotide sequence level or possibly the absence of an *ftsZ* gene altogether in these species.

The chromosomal DNAs from the gram-negative bacteria were digested with three other restriction endonucleases

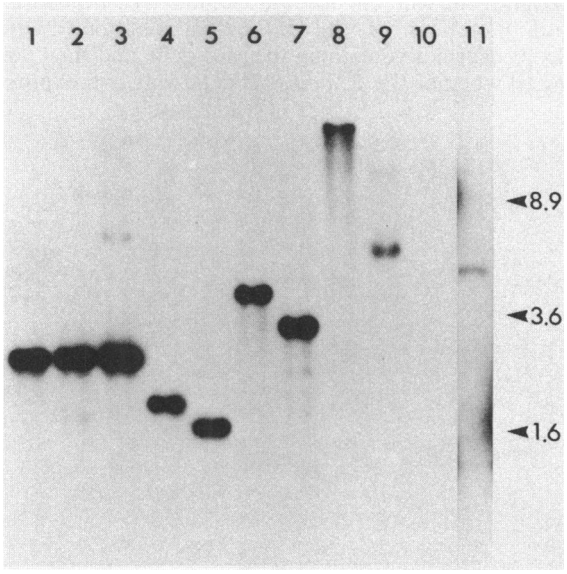

FIG. 2. Hybridization of the *ftsZ* gene to genomic digests of 11 species of bacteria. Chromosomal DNA from the various species was isolated, digested with *Eco*RI, and electrophoresed on an agarose gel. DNA in the agarose gel was transferred to GeneScreen Plus and hybridized with a nick-translated *ftsZ* gene probe. The molecular weight markers were determined from known fragments of DNA which contained the *ftsZ* gene. The gel contained DNA from the following organisms: lane 1, *E. coli*; lane 2, *C. diversus*; lane 3, *Shigella sonnei*; lane 4, *Serratia marcescens*; lane 5, *Salmonella typhimurium*; lane 6, *K. pneumoniae*; lane 7, *Enterobacter aerogenes*; lane 8, *Proteus mirabilis*; lane 9, *Pseudomonas aeruginosa*; lane 10, *Bacillus subtilis*; and lane 11, *A. tumefaciens*.

TABLE 2. Summary of chromosomal fragments which contain *ftsZ* sequences

Organism	Size (kb) of restriction fragments hybridizing to *ftsZ* gene probe [a]:			
	*Pst*I	*Bam*HI	*Eco*RI	*Hin*dIII
Escherichia coli	4.5	13.5	2.5	3.5
Citrobacter diversus	4.5	9.2	2.5	3.5
Shigella sonnei	*	13	2.5	3.7
Serratia marcescens	4.3	4.3	1.9	5.1
Salmonella typhimurium	4.1	>20	1.6	5.8
Klebsiella pneumoniae	2.4	>16	4.0	7.0, 9.5
Enterobacter aerogenes	2.3	9.0	3.0	>13 (11)
Proteus mirabilis	1.2	>20	>20	5.5
Pseudomonas aeruginosa	1.2	4.5	5.5	>20
Agrobacterium tumefaciens	ND	ND	4.6	3.5

[a] *, *Shigella sonnei* chromosomal DNA did not digest with *Pst*I. ND, Not determined. Number in parentheses represents a fragment which hybridized weakly to the *ftsZ* gene probe.

and, after transfer, were hybridized to the *ftsZ* gene probe. The number and sizes of these fragments combined with the results from Fig. 2 are summarized in Table 2. The *ftsZ* genes from *C. diversus* and *Shigella sonnei* were located on restriction fragments corresponding in size to the *E. coli* fragment containing the *ftsZ* gene, for at least three out of the four restriction enzymes tested. Since these restriction sites lay within the coding sequences of the genes which flank *ftsZ* (Fig. 1), conservation of the order of these genes was indicated. A number of species had their *ftsZ* genes on only one restriction fragment which was the same size as the corresponding fragment from *E. coli*; these species included *Serratia marcescens*, *Salmonella typhimurium*, *Enterobacter aerogenes*, and *A. tumefaciens*. All of the restriction fragments from *K. pneumonia*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* that hybridized to *ftsZ* differed in size from the corresponding fragments from *E. coli*.

FtsZ antigenic homology in gram-negative and gram-positive bacteria. Southern hybridization experiments indicated that all gram-negative, rod-shaped bacteria contain an *ftsZ* gene. To determine the nature of the FtsZ protein encoded by these genes, whole-cell lysates from these bacteria were examined by Western blot analysis. The *E. coli* FtsZ protein has been purified to homogeneity and used to produce antisera against the FtsZ protein (J. Ward and J. Lutkenhaus, manuscript in preparation). The immunoreactive proteins from eight members of the family *Enterobacteriaceae* and from *Pseudomonas aeruginosa* and *A. tumefaciens* are shown in Fig. 3A and B. All of the *Enterobacteriaceae* contained FtsZ-like proteins which were similar in molecular weight and immunoreactivity to FtsZ from *E. coli*. The molecular weight of the FtsZ from *S. marcescens* differed the most from the others (Fig. 3A, lane 4). *Pseudomonas aeruginosa* and *A. tumefaciens* had two detectable immunoreactive proteins. The major immunoreactive protein in *Pseudomonas aeruginosa* was slightly larger than the *E. coli* protein; the other less-reactive protein had a much greater molecular weight. The major immunoreactive protein in *A. tumefaciens* had a much greater molecular weight than the *E. coli* protein, whereas the slightly less-reactive protein had a molecular weight less than FtsZ. There are two ways to explain why *Pseudomonas aeruginosa* and *A. tumefaciens* contained proteins which did not immunostain to the same extent as the FtsZ proteins from more-related species. Either there was less FtsZ protein in these species or, more likely, the proteins were less im-

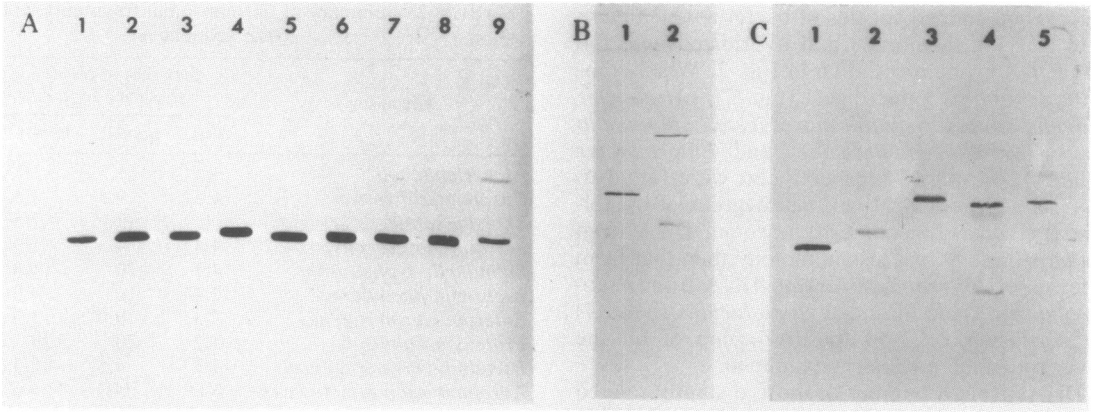

FIG. 3. Identification of FtsZ-related proteins from gram-negative and gram-positive bacteria by indirect immunological staining. Approximately equal amounts of cell lysates were applied to the 10% SDS-polyacrylamide gels. After electrophoresis, the proteins were transferred to nitrocellulose, and the immunoreactive proteins were stained by using the anti-FtsZ antiserum. (A) FtsZ-like proteins from gram-negative, rod-shaped bacteria. The order of lanes 1 through 9 is the same as lanes 1 through 9 identified in the legend to Fig. 2. (B) FtsZ-like proteins from *A. tumefaciens*. Lane 1, *E. coli*; lane 2, *A. tumefaciens*. (C) FtsZ-like proteins from species distantly related to *E. coli*. Lanes: 1, *E. coli*; 2, *Bacillus subtilis*; 3, *Streptococcus faecalis*; 4, *Staphylococcus aureus*; 5, *Branhamella catarrhalis*.

munoreactive because of a greater divergence from the *E. coli* protein at the amino acid sequence level.

As shown above, DNA from the gram-negative, coccal-shaped bacterium and the gram-positive bacteria had no nucleotide sequence homology with the *ftsZ* gene probe. To determine whether an FtsZ-like protein exists in these bacteria, lysates were also probed with the anti-FtsZ antisera. *B. subtilis* had one immunoreactive protein which had a molecular weight a little greater than that of *E. coli* protein, as shown in Fig. 3C, lane 2. All of the coccal-shaped bacteria, *Streptococcus faecalis* (Fig. 3C, lane 3), *Staphylococcus aureus* (lane 4), and *Branhamella catarrhalis* (lane 5), contained one major immunoreactive protein and at least one other minor band. The major immunoreactive proteins were significantly larger than the FtsZ proteins from the gram-negative and gram-positive rod-shaped bacteria, except for the major protein from *A. tumefaciens*. The proteins from these three and other coccal-shaped bacteria, including *M. luteus*, *Neisseria lactamica*, and *Neisseria perflava* (data not shown), had very similar molecular weights and may represent a class of FtsZ proteins unique to coccal-shaped bacteria.

Function and expression of the *E. coli ftsZ* gene in other *Enterobacteriaceae*. The evolutionary distance at which the *E. coli ftsZ* gene can function was examined by transforming various members of the *Enterobacteriaceae* with a multicopy plasmid containing the *ftsZ* gene and then determining (i) whether the *E. coli ftsZ* gene was overexpressed

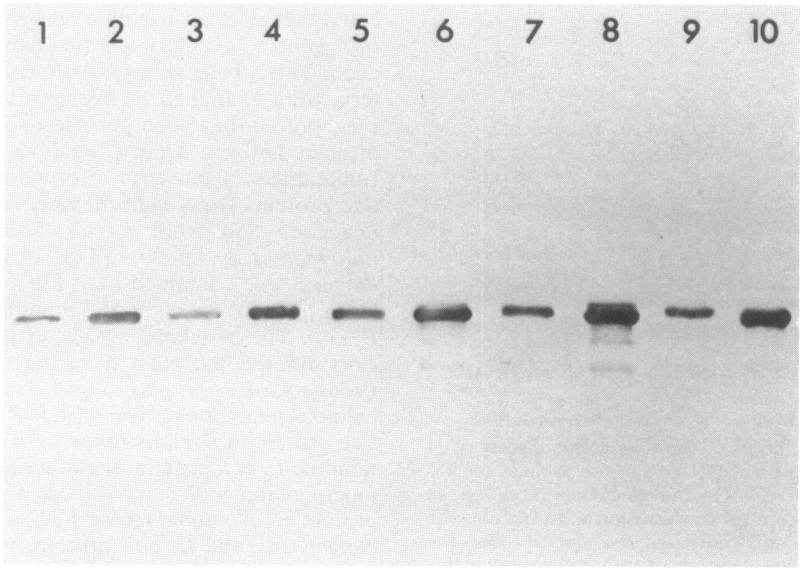

FIG. 4. Five *Enterobacteriaceae* species transformed with a plasmid containing the *E. coli ftsZ* gene overproduced FtsZ protein. Cell cultures of bacteria transformed with pZAQ were grown to the exponential phase, centrifuged, and suspended in SDS-sample buffer. Approximately equal amounts of the whole-cell lysates were electrophoresed with equal amounts of whole-cell lysates from the same species without the plasmid. The gel was processed as described in the legend to Fig. 3. Odd-numbered lanes, Lysates from cells without pZAQ; even-numbered lanes, lysates from cells containing pZAQ. Lanes: 1 and 2, *E. coli*; 3 and 4, *Shigella sonnei*; 5 and 6, *Salmonella typhimurium*; 7 and 8, *K. pneumoniae*; 9 and 10, *Enterobacter aerogenes*.

and (ii) whether the transformed organisms exhibited the minicell phenotype described previously (26). The minicell phenotype observed previously was induced by an increased level of FtsZ. Four *Enterobacteriaceae* were transformed with pZAQ. Two additional members, *Serratia marcescens* and *Proteus mirabilis*, which are resistant to tetracycline, were transfected with plasmid pKZAQ, a kanamycin-resistant, tetracycline-sensitive derivative of pZAQ. Microscopic examination of growing cells revealed that *Shigella sonnei, Salmonella typhimurium, K. pneumoniae*, and *Enterobacter aerogenes* exhibited the minicell phenotype but that *Serratia marcescens* and *Proteus mirqbilis* did not. Minicells were not observed in cultures of the plasmid-free strains.

Western blot analyses of lysates of the transformed species showed that the four *Enterobacteriaceae* species with the minicell phenotype overproduced FtsZ to an even greater extent than did *E. coli* containing pZAQ (Fig. 4). Furthermore, in *Salmonella typhimurium*, and *K. pneumoniae*, similar patterns of breakdown products of FtsZ could be seen. The two species that did not exhibit the minicell phenotype did not show an elevated level of FtsZ (data not shown).

Plasmid DNA from kanamycin-resistant colonies of *Serratia marcescens* and *Proteus mirabilis* were extracted and used to transform strain LE392. Kanamycin-resistant LE392 cells were shown to produce minicells and to contain a plasmid identical in mobility to pKZAQ. Lysates from *Serratia marcescens* and *Proteus mirabilis* containing pKZAQ were examined by Western analysis for the presence of the slightly smaller *E. coli* FtsZ protein. Only the chromosomal FtsZ protein could be detected. Thus, even though the intact *E. coli ftsZ* gene was present in these two strains, there was not enough *E. coli* FtsZ protein to be detected by immunostaining or to induce minicells.

The minicells produced by the four different species containing pZAQ were isolated by using sucrose density gradients and were labeled with [^{35}S]methionine for 1 h at 37°C. The results are shown in Fig. 5. Labeled minicells isolated from *E. coli* W3110 with pZAQ were run as a control (lane 1). The major labeled protein in each minicell preparation was FtsZ. Above this band was the cell division protein FtsA. The other gene encoded by the bacterial insert in pZAQ is *ftsQ*, but the product of this gene could not be identified on this autoradiogram and has not yet been identified. The fact that proteins which were encoded by the plasmid pZAQ were primarily labeled demonstrates that minicells from these four different species of *Enterobacteriaceae* containing the *E. coli ftsZ* gene could be isolated and used to analyze gene products encoded by plasmids. These results also suggest that the *E. coli* FtsZ protein was functional in these species.

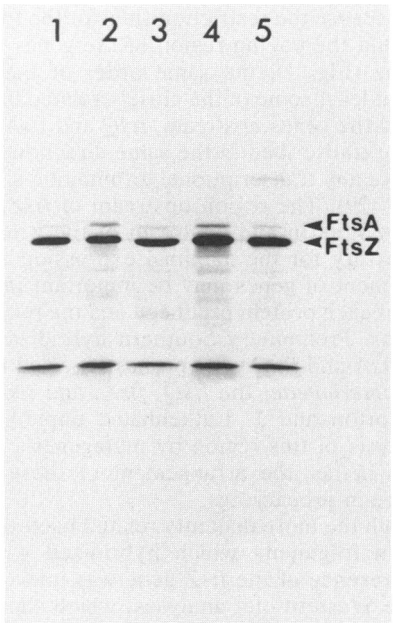

FIG. 5. Autoradiogram of polyacrylamide gel showing [^{35}S]methionine-labeled proteins synthesized in minicells isolated from five different species containing pZAQ. Minicells from the transformed strains were isolated and labeled for 1 h in the presence of [^{35}S]methionine, spun down, and then suspended in SDS-sample buffer for electrophoresis. Labeled samples were electrophoresed on a 10% polyacrylamide gel. The gel was dried and exposed to X-ray film. Lanes: 1, *E. coli*; 2, *Shigella sonnei*; 3, *Salmonella typhimurium*; 4, *K. pneumoniae*; 5, *Enterobacter aerogenes*.

DISCUSSION

Recent evidence suggests that the *ftsZ* gene controls a pivotal step in the cell division pathway. It appears to be an essential gene (16) involved in an early (2), rate-limiting (26) step in the formation of the cell division septum. It is also the step at which cell division is inhibited during the SOS response. If the FtsZ protein has a fundamental role in the cell division process, we would expect the protein to be found universally among procaryotes. This study has provided evidence that the *ftsZ* gene or FtsZ protein, or both were present in most if not all bacterial species, including rod- and coccal-shaped, gram-negative and gram-positive bacteria.

DNA homologous to the *ftsZ* gene from *E. coli* was found in seven members of the family *Enterobacteriaceae* and in *Pseudomonas aeruginosa* and *A. tumefaciens* (Fig. 2). The hybridization was very strong for the *Enterobacteriaceae* but was weaker for species from other families. Strengths of hybridization occurred in the following order: six *Enterobacteriaceae* > *Proteus mirabilis* > *Pseudomonas aeruginosa* > *A. tumefaciens*. DNA from *Branhamella catarrhalis, Bacillus subtilis, Staphylococcus aureus*, and *M. luteus* did not contain sequences which hybridized to our *ftsZ* gene probe, even under washing conditions of very low stringency.

The diversity of species containing sequences which hybridized in this study directly paralleled the results of a study in which the *rpsA* gene, the structural gene for the essential ribosomal protein S1, was compared in 10 bacterial species (23). Members of the *Enterobacteriaceae* showed strong hybridization, *A. tumefaciens* showed only weak hybridization, and gram-positive species did not hybridize at all. The *recA* gene, which has a fundamental role in homologous recombination, has about the same divergence in its nucleotide sequence. *A. tumefaciens* and *Rhizobium meliloti* contain DNA sequences which hybridize weakly to an *E. coli recA* gene probe (3).

The *ftsZ* genes from a number of bacterial species were located on restriction fragments of the same size as the corresponding fragment from *E. coli* (Table 2). *C. diversus* and *Shigella sonnei* contained the *ftsZ* gene on restriction fragments of the same size as that of *E. coli* for three out of the four restriction enzymes tested, whereas *Serratia marcescens, Salmonella typhimurium, Enterobacter aerogenes*, and *A. tumefaciens* had only one fragment the same size as that of *E. coli*. The other bacterial species examined had no *ftsZ* fragments with sizes similar to those of the *E. coli*

fragments. Because the restriction sites for the four enzymes used lay within the coding region of the genes which flank the *ftsZ* gene (Fig. 1), the same order of these genes is indicated in at least some of the closely related bacteria. The *ftsZ* gene and the genes upstream, *ftsQ* and *ftsA*, are tightly clustered, are transcribed in the same direction, and do not appear to have any transcriptional termination sites between them (20, 28, 29). The region upstream of *ftsZ*, containing putative promoters in and upstream of the genes *ftsQ* and *ftsA*, is necessary for the maximal expression of *ftsZ* (29). This arrangement of genes may be important in controlling the amount of each protein produced and the relative ratio of these proteins. Preliminary Southern hybridization experiments with *ftsA* and *ftsQ* gene probes revealed that, among the *Enterobacteriaceae*, the *ftsQ*, *ftsA*, and *ftsZ* genes are linked (J. Corton and J. Lutkenhaus, unpublished data). Further analysis of this region by mutagenesis and cloning will reveal whether the arrangement of these genes is a salient feature in procaryotes.

Even though the more distantly related bacteria contained no restriction fragments which hybridized with the *ftsZ* probe, the presence of the *ftsZ* gene was inferred from the results of the Western blot analyses, which showed that all species examined contained a major immunoreactive protein (Fig. 3A, B, and C). The bacteria examined in this study could be grouped into classes by the amount of antigenic homology of their FtsZ proteins to *E. coli* FtsZ, which was based on band intensity and the assumption that all species have the same amount of FtsZ. The amount of antigenic homology directly paralleled the amount of DNA sequence homology as follows: *Enterobacteriaceae* > *Pseudomonas aeruginosa* = *A. tumefaciens* > *Bacillus subtilis* = *Branhamella catarrhalis* = *Staphylococcus aureus* = *Streptococcus faecalis*.

All of the *Enterobacteriaceae* contained single proteins that were antigenically homologous to the FtsZ of *E. coli* and were very similar in molecular weight. The more distantly related gram-negative, rod-shaped species, *A. tumefaciens* and *Pseudomonas aeruginosa*, contained two immunologically cross-reactive proteins. In *Pseudomonas aeruginosa* the major cross-reacting band was similar in molecular weight to the band seen in the *Enterobacteriaceae*; however, in *A. tumefaciens* the cross-reactive bands were both dissimilar in molecular weight. A single cross-reactive band was seen in *Bacillus subtilis* which was slightly larger than the *E. coli* band. The coccal-shaped bacteria all contained cross-reactive bands that were much larger than the *E. coli* protein but similar in molecular weight to each other. These may have been FtsZ proteins with functions unique for coccal-shaped bacteria. Isolation and characterization of the *ftsZ* genes from the more distantly related species will be necessary to confirm that they are related to the *ftsZ* gene from *E. coli*. Characterization of these genes as well as the more closely related genes should prove very interesting in the study of the evolution and function of the *ftsZ* gene.

We wished to determine whether the *E. coli ftsZ* gene can be expressed in other species of bacteria and whether the FtsZ protein is functional. *E. coli* cells which harbor a multicopy plasmid encoding the *ftsZ* gene overproduce the FtsZ protein and exhibit the minicell phenotype (26). If the FtsZ protein of *E. coli* is overproduced in other bacteria, the production of minicells would be indicative of the function of the protein. Species from *Enterobacteriaceae* genera transformed with pZAQ overproduced FtsZ protein as shown by indirect immunostaining (Fig. 4). Microscopic analysis of these strains revealed that they were producing minicells, indicating that the plasmid-encoded *ftsZ* gene in these strains was functional. These minicells could be isolated by using sucrose density gradients, labeled with [^{35}S]methionine, and could be shown to synthesize plasmid-encoded proteins (Fig. 5).

Plasmids containing foreign genes can be analyzed in a minicell-producing *E. coli* strain carrying the *minB* mutation (1, 5). A more ideal situation would be to analyze plasmid-encoded proteins in the species from which the cloned genes originated. This would be especially necessary when there is a region on the cloned DNA fragment controlling gene expression which requires regulatory proteins unique to the organism. The use of multicopy plasmids containing the *ftsZ* gene from *E. coli* should allow the analysis of plasmid-encoded gene products in closely related strains for which a minicell-producing mutant does not exist. Alternatively, cloning the *ftsZ* gene from a species and reintroducing it into the same species under conditions of increased expression might result in minicell production.

E. coli cells containing pZAQ produced minicells but otherwise appeared normal. In contrast, the population of transformed cells from the other four *Enterobacteriaceae* members contained lysed and deformed cells. This effect appeared more pronounced as the evolutionary distance of the species from *E. coli* increased. Thus, *Shigella sonnei* and *Enterobacter aerogenes* were not affected to the same degree as *Salmonella typhimurium* and *K. pneumoniae*. This difference was most probably caused by overproduction of the FtsZ protein, although it could have been caused by FtsA or FtsQ as well. If the *E. coli ftsZ* gene at a high copy number is deleterious to non-*E. coli* cells which harbor pZAQ, then *E. coli* cells containing the *ftsZ* gene from another species might act in a similar manner. This might explain why we could not complement a temperature-sensitive *ftsZ* mutant with libraries of chromosomal DNA from various *Enterobacteriaceae* cloned into pBR322 (Corton and Lutkenhaus, unpublished data).

Proteus mirabilis and *Serratia marcesens* did not display the minicell phenotype upon transfection with the multicopy *ftsZ* plasmid. Since these species did not overproduce the *E. coli* FtsZ protein, this result was not surprising, but it left open the question of whether the *E. coli* FtsZ protein would function in these species if overexpressed. Apparently the transcriptional signals for the *E. coli ftsZ* gene must not have been efficiently recognized in these species, or the *E. coli* FtsZ protein was unstable.

This study suggests that the *ftsZ* gene is present in all bacterial species. Earlier evidence suggested that the *ftsZ* gene is a key component of the SOS-induced filamentation response since it is the sole target of the cell division inhibitor SulA, which is induced during the SOS response. If the SOS response exists in other bacteria and if there is such a response to DNA damage, does the response involve inhibition of cell division by the inhibition of FtsZ by SulA? Evidence suggests that SOS-like functions exist in such diverse organisms as *Bacillus subtilis* (12) and *Saccharomyces cerevisiae* (22). *E. coli recA* strains containing plasmids with cloned *recA* genes from *Shigella flexneri*, *Escherichia coli* B/r, *Erwinia carotovora*, or *Proteus vulgaris* are inducible with nalidixic acid, a DNA-damaging agent, and synthesize large amounts of the heterologous RecA protein. This indicates that the foreign *recA* genes are under the control of the *E. coli* LexA repressor (10) and that the foreign RecA proteins have retained their ability to cleave the *E. coli* LexA repressor. In vitro analysis of the purified RecA protein from *Bacillus subtilis* (13) and *Proteus mirabilis* (27) has shown

that these proteins have also retained an ability to cleave the *E. coli* LexA protein. Futhermore, the Lon protease, which appears to degrade SulA, is conserved in a wide variety of bacterial species (21). Recent evidence in our laboratory obtained by Southern hybridization indicates that the cell division inhibitor gene *sulA* is also conserved throughout the *Enterobacteriaceae* family (J. Corton and J. Lutkenhaus, unpublished observation). Such conservation of a nonessential gene suggests that division inhibition during the SOS response is important in the survival of these species.

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