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High-Level Expression of the FtsA Protein Inhibits Cell Septation in *Escherichia coli* K-12

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DNA fragments encoding the *ftsA* gene were subcloned into plasmids downstream of a *lac* promoter or a *tac* promoter. These plasmid constructs, when transformed into wild-type and mutant strains, inhibited normal cell septation, causing the formation of long nonseptate filaments. This phenotype is due to overproduction of the FtsA protein.

The *ftsA* and *ftsQ* genes, which are located in the vicinity of the 2-min region on the *Escherichia coli* K-12 genetic map, have been proven to be essential to cell division in this bacterium (1, 8, 9). The genes are closely linked in the order *ftsQ*, *ftsA*, and *ftsZ*. There are promoters upstream of each gene, but there are no strong transcription terminators between the genes (13, 14, 18, 22). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of FtsA protein that had been synthesized in vivo from a plasmid (4, 21, 22) or bacteriophage lambda template (12) indicated a molecular weight of 46,000 to 50,000. The nucleotide sequence of the *ftsA* gene has been determined (13, 22), and from these data a molecular weight of 45,300 has been predicted for the FtsA protein. DNA sequence analysis also predicted the FtsQ protein to have a molecular weight of 31,400 (12, 22). FtsQ product has recently been overproduced and the protein product has been observed on sodium dodecyl sulfate-polyacrylamide gels (17).

Based on the amount of protein labeled in vivo (4, 21) and from reporter gene analysis of the *ftsA* promoters (14, 22), the level of expression of the FtsA protein can be characterized as low. To enhance the level of FtsA protein synthesis to facilitate its purification, we subcloned a DNA fragment containing both *ftsA* and *ftsQ* genes into plasmids that increase transcription of these genes.

A 2.2-kilobase (kb) *Eco*RI DNA fragment from plasmid pZAQ, encoding both *ftsA* and *ftsQ* (21), was purified from a low-gelling, low-melting-temperature agarose gel (Seaplaque; FMC Marine Colloids) (16) and was initially subcloned into expression vector pBH20 (Fig. 1). pBH20 is a pBR322 plasmid derivative that contains a *lac* promoter near the *Eco*RI restriction site (11). Recombinant plasmids containing the *ftsA* gene were isolated from carbenicillin-resistant transformant cells of YC100, a temperature-sensitive conditional *ftsA10* mutant (4), that grew at the nonpermissive temperature (42°C). These YC100 transformants exhibited two colony phenotypes on yeast extract-tryptone (10) agar plates containing 25 μg of carbenicillin per ml: glistening colonies of large diameter and opaque colonies of small diameter. Restriction enzyme digestions of plasmid DNA and analysis by agarose gel electrophoresis demonstrated that plasmid DNA isolated from cells producing the large colonies had the 2.2-kb *Eco*RI fragment in an orientation inappropriate for *lac* promoter expression, while plasmid DNA isolated from cells producing the small colonies contained the 2.2-kb fragment in the correct orientation for *lac* promoter expression of the *ftsA* and *ftsQ* genes. The latter plasmid was designated pLHW1 (Fig. 1).

Observation of YC100 cells containing pLHW1 with a light microscope showed that the cells were inhibited in cell division. Cells isolated from either a bacterial colony or broth cultures of yeast extract-tryptone medium appeared primarily as long filaments, although some cells of normal length and short filaments were also present (Fig. 2B). Introduction of pLHW1 into wild-type *E. coli* AMC290 (4) resulted in the same phenotype as displayed in strain YC100. Colonies of AMC290 carrying pLHW1 were much smaller in diameter at 37°C than colonies of AMC290 or AMC290 (pBH20). Microscopic observation of AMC290(pLHW1) revealed long filamentous cells. Even when pLHW1 was transformed into the *lacI*^{q} strain JM101, in which transcription from the *lac* promoter should be considerably inhibited, the cells were filamentous, though not to the same extent as in the case of AMC290.

We also subcloned the 2.2-kb *Eco*RI fragment into another expression vector, pKK223-3 (Pharmacia LKB) (3) (Fig. 1). This vector contains a *tac* promoter plus strong rRNA transcription terminators downstream from a multicloning site. Plasmid DNA was isolated from transformant cells of a *lacI*^{q}-containing strain (JM101) that was unable to grow on yeast extract-tryptone agar medium containing 10^{-3} M isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma Chemical Co.). This plasmid, with the 2.2-kb *Eco*RI fragment in the correct orientation for *tac* expression of *ftsA* and *ftsQ* (Fig. 1), was designated pLHW2. Transformants of pLHW2 DNA could not be obtained in either the *ftsA* mutant (YC100) or wild-type (AMC290) bacteria, presumably due to lethal effects resulting from uncontrolled expression of the *ftsA* gene. Microscopic examination of JM101 cells containing pLHW2 once again revealed their morphology to be long filaments.

Cell filament formation induced by plasmids pLHW1 and pLHW2 could be due to overproduction of either FtsA or FtsQ proteins. Two additional plasmids were constructed to test the hypothesis that overexpression of FtsA alone is the direct cause of the phenotype. The first construct was made by deleting a 0.5-kb *Hin*dIII DNA fragment encoding the

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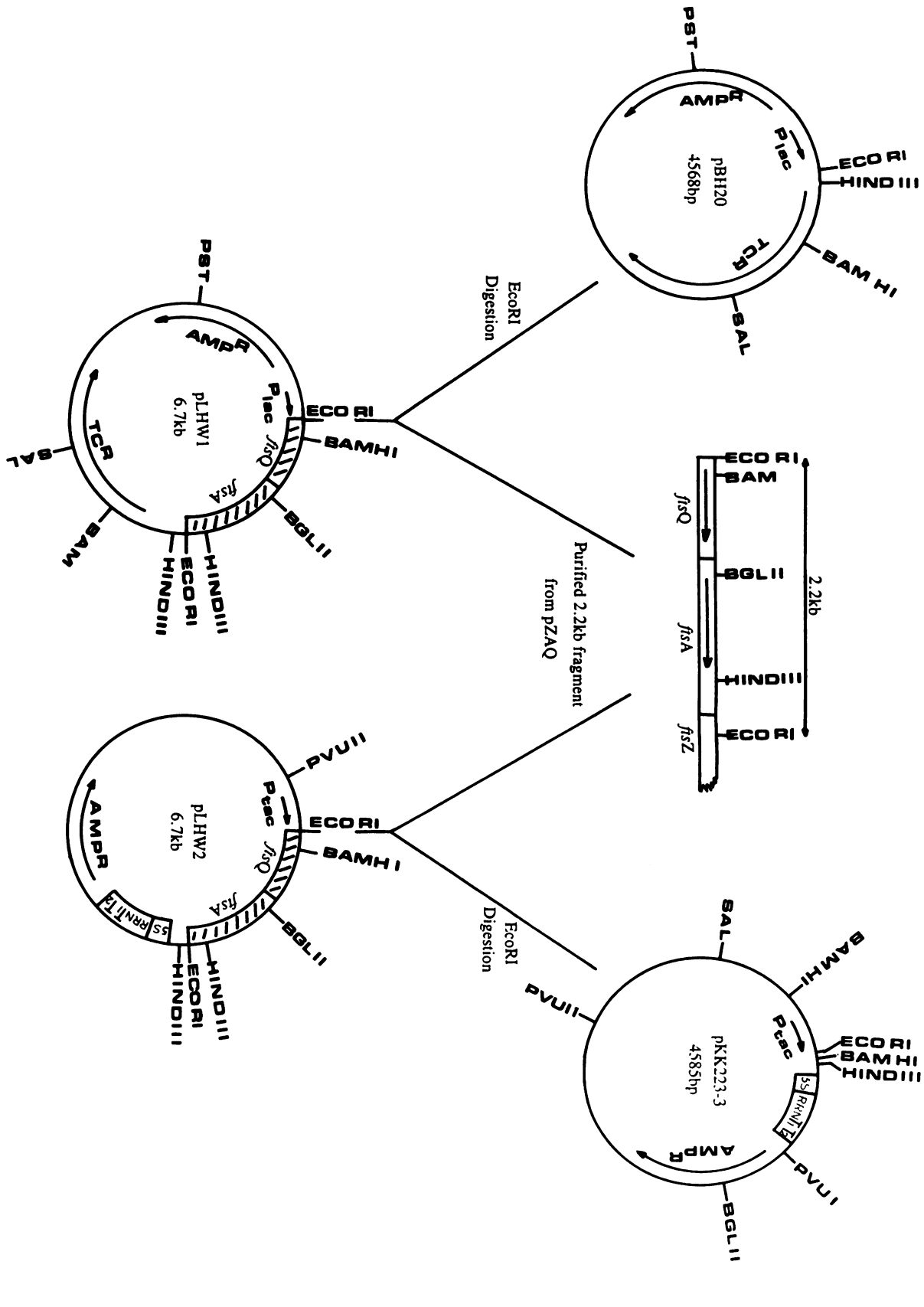

FIG. 1. Schematic diagrams of plasmids and plasmid constructions of pLHW1 and pLHW2. The hatched band represents the 2.2-kb *Eco*RI fragment containing *fisQ* and *fisA*; arrows indicate direction of transcription. Abbreviations: P_{lac}, *lac* promoter; P_{tac}, *trp-lac* promoter; Tc^r, tetracycline resistance; Amp^R, ampicillin resistance, 5S, rRNA; RRN T_1T_2, ribosomal terminator sequences; Bam, *Bam*HI; Sal, *Sal*I; Pst, *Pst*I.

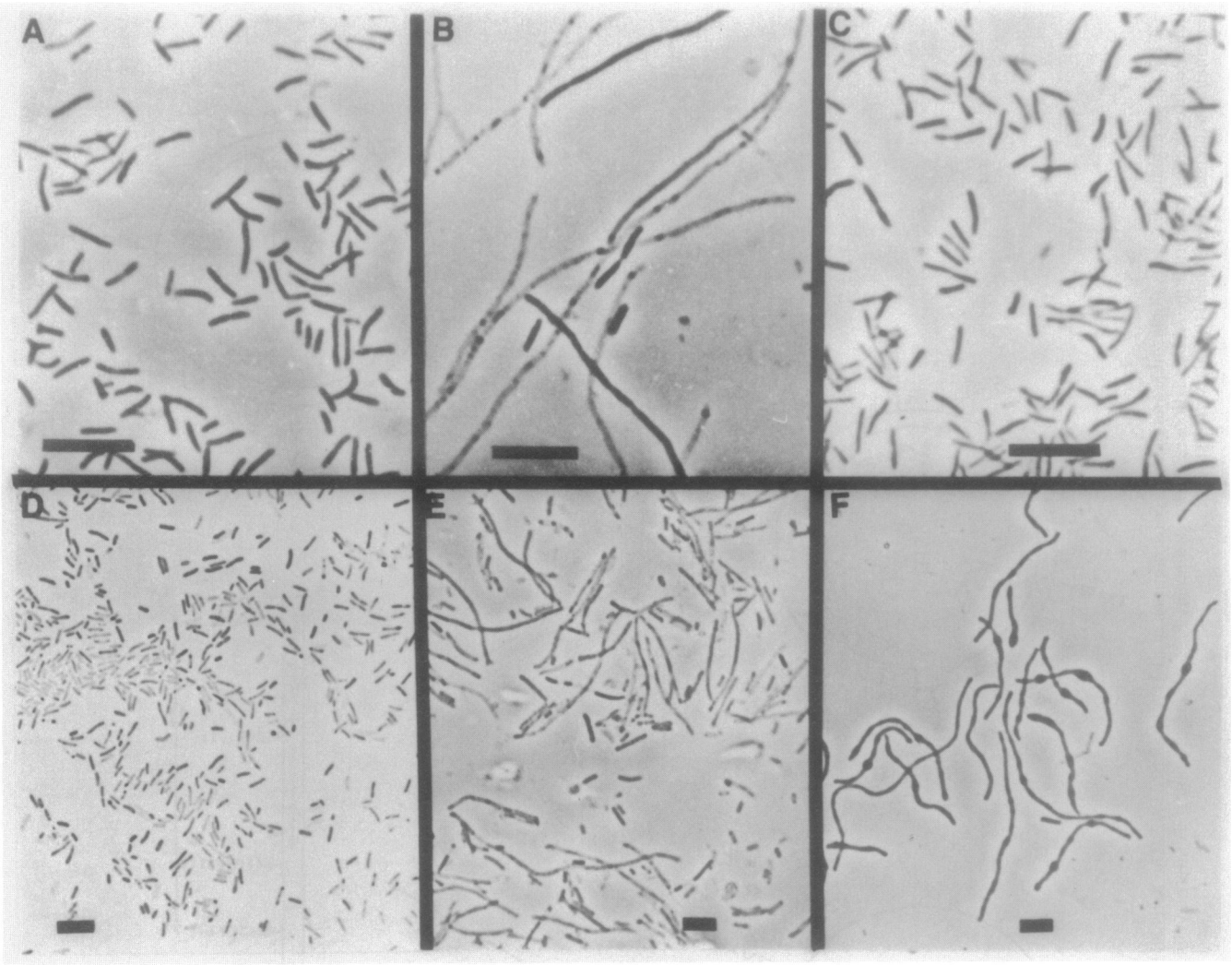

FIG. 2. Phase-contrast photomicrographs showing morphology of *E. coli* with and without plasmids. (A) YC100; (B) YC100(pLHW1); (C) YC100(pLHW2-1); (D) JM101; (E) JM101(pLHW3); (F) JM101(pLHW3) after 2 h with 10^{-4} M IPTG. Bar, 15 nm.

carboxy-terminal end of the FtsA protein from pLHW2 (Fig. 1). The resulting plasmid was designated pLHW2-1. Transformant cells of JM101, AMC290, and YC100 that contained plasmid pLHW2-1 appeared exclusively as uniform short rods upon microscopic examination (Fig. 2C).

The second construct was made by subcloning the 2.4-kb *Bam*HI fragment from pLHW1 into the *Bam*HI site downstream of the *tac* promoter in plasmid pKK223-3. This *Bam*HI DNA fragment encodes an intact *ftsA* gene, but only a 0.1-kb truncated *ftsQ* gene. The resulting plasmid, designated pLHW3, was isolated from a transformant of an *ftsA* *lacI*^{q} strain, RGL201, that grew at the nonpermissive temperature. Strain RGL201 was constructed by introduction of the Tn*5tacI* transposon (5) into strain YC100 to provide a *lacI*^{q} gene. Similarly, the inactivation of the *ftsQ* gene in plasmid pLHW3 was confirmed by utilizing an *ftsQ* mutant containing a *lacI*^{q} gene, RGL202. RGL202 had been constructed by Tn*5tacI* transposition into the *ftsQ* mutant, TOE1 (2). pLHW3 DNA transformants of RGL202 failed to grow at the nonpermissive temperature of 42°C, whereas pLHW2 transformants grew at 42°C. This confirmed that pLHW3 did not specify the *ftsQ* gene product.

Microscopic examination of RGL201 and JM101 cells containing plasmid pLHW3 again revealed their morphology to be long filaments (Fig. 2E). Moreover, strains containing either pLHW2 or pLHW3 developed large spherical bulges at the filaments (Fig. 2F) after 2 h of increased transcription induced by 10^{-4} M IPTG. This observation suggests that normal cell elongation may also be affected when FtsA is highly expressed.

An additional plasmid construct, designated pLHW4, was made by subcloning the 0.5-kb *Hin*dIII fragment from pLHW1 into the *Hin*dIII site in plasmid pKK223-3 in the correct orientation. The *Hin*dIII fragment is the deleted DNA fragment used to damage the *ftsA* gene of plasmid pLHW2-1. This DNA fragment encodes not only the carboxyl end of the FtsA, but also the first 37 N-terminal amino acids of FtsZ. Overproduction of this FtsZ peptide fragment could conceivably act as a competitor of wild-type product and result in filamentation. Microscopic examination of JM101 cells containing pLHW4 indicated uniform short rods even in the presence of 10^{-3} M IPTG. Thus, there is no apparent effect caused by overexpressing the *ftsZ* gene fragment.

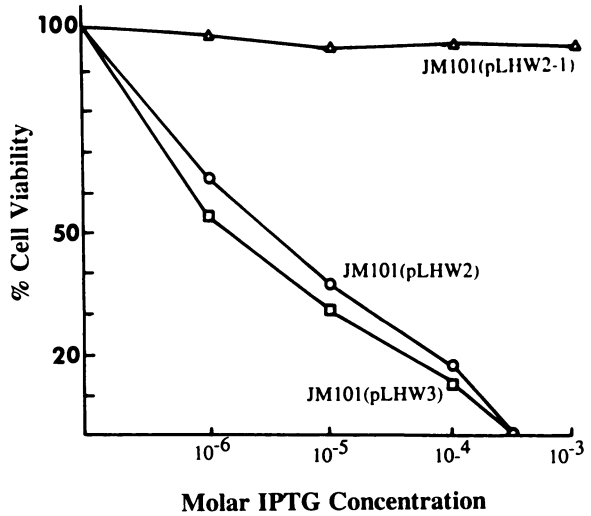

FIG. 3. Cell survival of JM101(pLHW2), JM101(pLHW3), and JM101(pLHW2-1) on complex agar plates with various IPTG concentrations.

To test the IPTG sensitivity of JM101 cells containing plasmid pLHW2, pLHW3, or pLHW2-1, inocula from exponentially growing cultures were spread on yeast extract-tryptone agar plates containing 0 to 10^{-3} M IPTG. Figure 3 displays how survival of JM101(pLHW2) and JM101 (pLHW3) cells decreased as a linear function of increasing IPTG concentration. JM101(pLHW2-1) cells (Fig. 3), as well as JM101(pKK223-3) cells (data not shown), were not killed at any concentration of IPTG.

Maxicell labeling of the proteins expressed from plasmids pLHW1 and pLHW2-1 in *E. coli* CSR603 (15) were performed (data not shown). Transformants of CSR603 carrying pLHW2 or pLHW3 could not be isolated. A band of FtsA protein strongly labeled with [^{35}S]methionine was recovered from maxicells containing pLHW1. Bands putatively representing truncated FtsA and FtsQ protein products were labeled in maxicells containing pLHW2-1. Labeled FtsQ protein was not recovered from maxicells containing pLHW1.

A recent paper by Storts et al. (17) did not report any phenotypic alteration resulting from overproduction of FtsQ protein. Their observation is consistent with the morphology of cells carrying plasmid pLHW2-1 with a deletion in the *ftsA* gene. Although a contribution from FtsQ is possible, our data suggest that the inhibition of cell septation is due to the overproduction of the FtsA protein. A similar inhibition of cell division, due to a high level of FtsA protein, was observed by Dewar et al. (7) when they constructed a high-copy vector containing the *ftsQ* and *ftsA* coding sequence, pSZ24. Elevated amounts of FtsA protein could be inhibiting cell septation in several possible ways, including (i) preventing formation of a required complex of septum proteins (4, 8, 19, 20); (ii) preventing "activation" of itself or other proteins by inhibition of some critical enzyme (A. C. Robinson, J. F. Collens, and W. D. Donachie, letter, Nature [London] **328**:766, 1987); or (iii) interfering with proper gene expression of other cell septation proteins (8). The observation that both alteration and overexpression of FtsA result in inhibition of cell division suggests the simple hypothesis that specific interactions of the FtsA protein and other division proteins are required for the septation process. Altered or overexpressed FtsA disrupts or distorts these septa-protein complexes.

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