

# Role of the *Escherichia coli* SurA Protein in Stationary-Phase Survival

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**SurA is a periplasmic peptidyl-prolyl isomerase required for the efficient folding of extracytoplasmic proteins. Although the *surA* gene had been identified in a screen for mutants that failed to survive in stationary phase, the role played by SurA in stationary-phase survival remained unknown. The results presented here demonstrate that the survival defect of *surA* mutants is due to their inability to grow at elevated pH in the absence of  $\sigma^S$ . When cultures of *Escherichia coli* were grown in peptide-rich Luria-Bertani medium, the majority of the cells lost viability during the first two to three days of incubation in stationary phase as the pH rose to pH 9. At this time the surviving cells resumed growth. In cultures of *surA rpoS* double mutants the survivors lysed as they attempted to resume growth at the elevated pH. Cells lacking penicillin binding protein 3 and  $\sigma^S$  had a survival defect similar to that of *surA rpoS* double mutants, suggesting that SurA foldase activity is important for the proper assembly of the cell wall-synthesizing apparatus.**

Bacteria have evolved a multitude of starvation-inducible mechanisms that lead to profound changes in cellular morphology and physiology (25, 40, 42). Induction of these changes results in cells with a greatly increased ability to survive many different environmental stresses, including starvation itself (26). Another survival strategy that appears to be quite widespread in the microbial world is a phenomenon we have termed GASP (for growth advantage in stationary phase) (45–47). When bacterial cultures are incubated for prolonged periods in stationary phase under many different conditions, there can be a dramatic loss in the number of CFU. Surprisingly, the survivors almost invariably are mutants able to grow at the time that the majority of the cells lose viability. The GASP phenomenon is easily observed when cultures of *Escherichia coli* K-12 are incubated in Luria-Bertani (LB) medium at 37°C. After reaching an initial titer of approximately  $5 \times 10^9$  CFU/ml after overnight incubation, titers drop during the first week and then stabilize at approximately  $10^8$  CFU/ml (47). The surviving cells are invariably mutants expressing the GASP phenotype, which can be assessed by their ability to take over a wild-type strain in mixed cultures during stationary-phase incubation.

In an effort to understand more about the surviving cells of *E. coli* cultures kept in stationary phase for long periods, we designed a screen for mutants unable to survive under these conditions and in this way discovered *surA* (43). Mutants that lacked SurA grew normally but completely lost viability after 3 to 5 days of incubation in rich medium. The nucleotide sequence of *surA*, determined later, indicated that SurA was similar in amino acid sequence to parvulin, a peptidyl-prolyl isomerase (PPIase) (37). Subsequently, it was determined that SurA was a periplasmic protein required for the proper assembly

of several outer membrane proteins (22) and that it possessed PPIase activity (29, 36).

We wondered how the loss of a periplasmic PPIase could result in a survival defect during stationary phase. *E. coli* possesses three periplasmic PPIases: PpiA (23), FkpA (16), and SurA (29, 36, 37). Mutants that lack PpiA or FkpA have no obvious phenotype (39). Therefore, the phenotype associated with the lack of SurA was very unusual. This difference in phenotype suggested that the functions of these PPIases are not entirely redundant. The differential, but interconnected, regulation of the three activities also suggested nonredundant functions. Both *ppiA* and *fkpA* are induced in response to periplasmic stresses (e.g., unfolded proteins or elevated pH) and are members of the CpxRA and  $\sigma^E$  regulons, respectively (5, 6, 31). The *surA* gene does not appear to be a member of either of these regulons, but its loss induces the expression of the  $\sigma^E$  regulon (5, 29, 31, 36). In an effort to understand why loss of this particular PPIase should cause cells to die in stationary phase, we further analyzed the properties of *surA* cells. The results of these analyses are presented here.

## MATERIALS AND METHODS

**Strains and growth conditions.** Our standard laboratory wild-type strain of *E. coli* is K-12 ZK126 (W3110  $\Delta lacU169 ina-2$ ) (4). Mutations were transduced into this strain with bacteriophage P1vir (28), and the resulting strains are described in Table 1. Cells were grown in LB medium and incubated at 37°C with aeration for the time periods indicated in the figures. Cultures were supplemented with kanamycin (50  $\mu$ g/ml) or tetracycline (15  $\mu$ g/ml) where appropriate.

**Microscopy.** Samples from cultures were spotted onto microscope slides coated with 0.8% agar and then viewed with a Nikon Optiphot-2 phase-contrast microscope at  $\times 60$  magnification. Images were collected with a charge-coupled device camera linked to a Macintosh 8600 computer with Scion Image software for image processing.

**Immunoblot detection of proteins.** Detection of SurA was performed as previously described (10) with rabbit polyclonal antiserum raised against SurA. This antiserum was obtained under our contract with Immuno-Dynamics Inc. (La Jolla, Calif.) with purified SurA (22). Log-phase cells were at an optical density at 600 nm ( $OD_{600}$ ) of 0.6, and stationary-phase cultures grew overnight.

**pH sensitivity assay.** To determine the effect of pH on growth, exponentially growing cultures ( $OD_{600}$ , 0.1) were resuspended into fresh prewarmed LB medium that had been either untreated or adjusted to pH 9 with NaOH. Growth was monitored by observing  $OD_{600}$ .

**High-pressure liquid chromatography (HPLC).** The composition of the murein sacculus was determined by following the protocol of Glauner (8). Cells from mid-log-phase cultures ( $OD_{600}$ , 0.7) were used for analysis.

**Penicillin binding protein (PBP) assay.** Cells were grown at 37°C in 10 ml of LB medium to an approximate  $OD_{600}$  of 1. Culture volumes were adjusted with LB medium as necessary to an  $OD_{600}$  of 1. Five milliliters of cells was pelleted

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TABLE 1. Strains used in this study

Strain	Relevant genotype	Reference or source
ZK126	W3110 $\Delta lacU169$ <i>tna-2</i>	4
ZK1000	ZK126 <i>rpoS</i> ::Km	3
ZK1171	ZK126 <i>rpoS</i> ::Tn10	24
SL238	ZK126 <i>rpoS</i> ::Tn10	This work
ZK819	ZK126 <i>rpoS819</i>	47
ZK880	ZK819 <i>surA1</i> ::mini-Tn10(Km)	43
SL133	ZK126 $\Delta surA3$ ::Km <i>rpoS</i> <sup>+</sup>	22
SL239	SL238 $\Delta surA3$ ::Km	This work
EC295	<i>ftsI23 leu</i> ::Tn10	11
SL268	ZK126 <i>ftsI23 leu</i> ::Tn10	This work
SL270	ZK1000 <i>ftsI23 leu</i> ::Tn10	This work

and resuspended in 125  $\mu$ l of spheroplast buffer (50 mM Tris [pH 8], 5 mM EDTA, 500 mM sucrose). Forty microliters of this suspension was transferred to a fresh tube and incubated for 10 min at 4°C. Then 2.6  $\mu$ l of 300  $\mu$ M fluorescein-hexanoic acid-6-amino penicillanic acid (Flu-Apa) (gift of D. Weiss) was added, and the mixture was incubated at 30°C for 30 min. An equal volume of 2 $\times$  loading buffer (1) was added, and cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were visualized and quantitated with a Fluorimager (Molecular Dynamics).

## RESULTS

**Characterization of *surA* mutant cells.** SurA appeared to be specifically required for survival during stationary phase (43). Thus, it made sense to investigate whether *surA* expression was growth phase regulated. To determine whether SurA was specifically induced in stationary phase and whether it was regulated by  $\sigma^S$ , the stationary-phase-specific sigma factor, we quantitated the abundance of SurA in a strain lacking  $\sigma^S$  (20). The results of Western blot analysis are shown in Fig. 1A. The amount of SurA was unchanged in an *rpoS*::Km strain relative to that in the wild type and did not change significantly when the cells were in stationary phase.

While  $\sigma^S$  does not control the expression of *surA*, we serendipitously discovered that the allelic state of *rpoS* greatly affects the expression of the survival (Sur<sup>-</sup>) phenotype. When the original *surA1*::mini-Tn10(Km) allele was transduced into a wild-type strain, we observed that the Sur<sup>-</sup> phenotype was lost, i.e., transductants survived in stationary phase like the wild type. Since it has been reported that many laboratory wild-type *E. coli* K-12 strains harbor mutant *rpoS* alleles (17, 18, 47), we tested the original *surA1*::mini-Tn10(Km) strain for the activity of  $\sigma^S$ . We determined that the original strain in which the *surA1*::mini-Tn10(Km) mutation was isolated did indeed contain a mutation in *rpoS* (*rpoS819*) and that this mutation was also required for the loss of viability in stationary phase. To further test the notion that in order to express the Sur<sup>-</sup> phenotype a strain needs mutations in both *surA* and *rpoS*, we constructed strains containing combinations of null alleles of these genes ( $\Delta surA3$ ::Km and *rpoS*::Tn10). Strains carrying null alleles of either *rpoS* or *surA* alone did not have noticeable defects in stationary-phase survival (Fig. 1B); only when mutant alleles of both genes were present did all the cells of the culture lose viability in stationary phase (Fig. 1B).

**Effect of pH on the survival of *surA rpoS* double mutants.** SurA is found in growing and nongrowing cells but is essential only during stationary phase. This finding suggests that particular changes in the environment during stationary phase are responsible for rendering SurA essential only during this phase. In order to understand why SurA function is required in the absence of  $\sigma^S$ , environmental changes that occur during stationary phase in LB medium cultures had to be determined.

Tormo et al. (43) observed that, unlike cells in LB medium, *surA1*::mini-Tn10(Km) *rpoS819* cells grown in minimal glucose medium were able to survive for a long time (20 days) during incubation in stationary phase. Since the main carbon source in LB medium is peptides and amino acids, these cultures become alkaline in stationary phase due to the release of excess amine-containing compounds. In contrast, the pH of M63-glucose-grown culture supernatants remains near neutrality (pH 6.5 to 7). To test whether the increase in pH in LB medium cultures was responsible for the loss of viability of *surA1*::mini-Tn10(Km) *rpoS819* mutants, 40 mM MOPS (morpholinepropanesulfonic acid; pH 7.4) was added to buffer the LB medium cultures. In this medium, *surA1*::mini-Tn10(Km) *rpoS819* cells did not lose viability (Fig. 2). That the Sur<sup>-</sup> phenotype is observed only in cells carrying both mutations indicates that a wild-type allele of either *surA* or *rpoS* is sufficient for cell survival in stationary phase at elevated pH.

Although SurA is present in exponentially growing cells, little if any  $\sigma^S$  is present during exponential phase when cells are grown in LB medium at 37°C. We therefore hypothesized that loss of SurA should also increase the sensitivity of exponential-phase cells to alkaline pH. To test this, exponentially growing cells of four strains, the wild type, a  $\Delta surA3$ ::Km strain, an *rpoS*::Tn10 strain, and a  $\Delta surA3$ ::Km *rpoS*::Tn10 strain, were resuspended in fresh LB medium adjusted to pH 9 with sodium hydroxide and incubated at 37°C (Fig. 3). For the first 30 min of incubation, all four strains continued to grow exponentially. After that time, the wild-type strain continued to grow, the *rpoS*::Tn10 strain lagged for a bit and then resumed growth to a level similar to that of the wild type, and the two

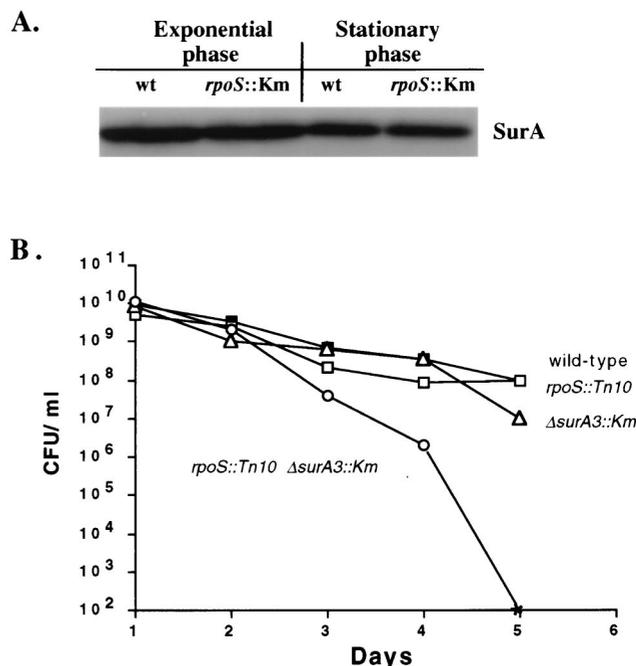


FIG. 1. (A) SurA levels are independent of  $\sigma^S$  and growth phase. The levels of SurA in wild-type (wt) and *rpoS*::Km cells were detected by immunoblot analysis in exponential-phase (OD<sub>600</sub>, 0.6) and stationary-phase (overnight) cells. (B) Mutations in both *rpoS* and *surA* are required to express the Sur<sup>-</sup> phenotype. Levels of viability of the wild type (filled squares), *rpoS*::Tn10 mutant (open squares),  $\Delta surA3$ ::Km mutant (open triangles), and *rpoS*::Tn10  $\Delta surA3$ ::Km double mutant (open circles) in LB medium are shown. The asterisk represents <10<sup>2</sup> CFU/ml. This experiment was repeated more than three times, and representative data are shown.

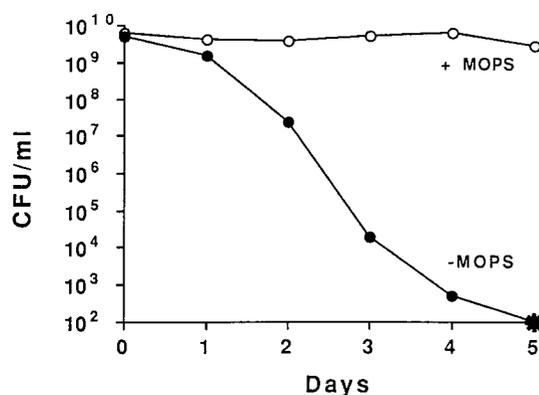


FIG. 2. Growth of the *surA1::mini-Tn10(Km) rpoS819* double mutant in LB medium (filled circles) or LB medium plus 40 mM MOPS (pH 7.4) (open circles). The asterisk represents  $<10^2$  CFU/ml. This experiment was repeated three times, and representative data are shown.

strains lacking SurA stopped growing and began to lyse (Fig. 3). Lysis was confirmed by microscopic observation (Fig. 4). These results demonstrate that SurA is required for growth at pH 9.

Both the  $\Delta surA3::Km$  and the  $\Delta surA3::Km rpoS::Tn10$  strain lysed in the experiment shown in Fig. 3, whereas in stationary phase the  $\Delta surA3::Km$  strain survived and only the double mutant died. During exponential growth the  $\Delta surA3::Km$  mutant strain should not contain much  $\sigma^S$ , since  $\sigma^S$  is not highly expressed during exponential phase in LB medium at 37°C. As cell growth slows in early-stationary-phase cultures, the  $\sigma^S$  regulon is induced, before the pH of the medium becomes alkaline. This suggests that  $\sigma^S$  expression can compensate for the loss of SurA.

The results shown in Fig. 3 demonstrate that SurA is required during exponential growth at elevated pH but do not answer the question of why both SurA and  $\sigma^S$  are required for long-term survival in stationary phase. The answer may lie in the fact that stationary-phase LB medium cultures are highly dynamic. As most of the cells died, GASP mutants took over the culture. For the first two days of incubation in stationary phase, the  $\Delta surA3::Km rpoS::Tn10$  strain lost viability, with kinetics similar to those of the wild type. The difference in levels of viability between the two cultures became apparent on the third to fourth days of incubation (Fig. 1B), the same period during which the GASP mutants grew and took over the culture (47). This suggests that either SurA or  $\sigma^S$  activity was specifically required for growth of the GASP mutants at elevated pH in these stationary-phase cultures. Consistent with the idea that SurA is required only for growth is the observation that after overnight incubation the nongrowing cells were completely viable although the medium was at pH 9 (Fig. 1B).

**Morphological changes in a  $\Delta surA3::Km rpoS::Tn10$  mutant.** Microscopic analysis of mutant cells in stationary phase was performed to determine whether the mutant cells had altered morphology. These experiments indicated that  $\Delta surA3::Km rpoS::Tn10$  mutant cells indeed had aberrant morphology (Fig. 5). The  $\Delta surA3::Km rpoS^+$  cells appeared similar to the wild-type cells in overnight cultures (data not shown). Two-day-old cultures of the wild-type and  $\Delta surA3::Km rpoS::Tn10$  strains also appeared similar (Fig. 5). After 4 days of incubation, the cell morphology became drastically altered. Many of the double-mutation cells were misshapen, having bulges in their middle sections. Other cells appeared extremely small (Fig. 5). Experiments with the vital stain acridine orange suggested that

the large bulging cells were the cells that were attempting to resume growth and that the small cells had lost viability (data not shown). Decrease in the OD of the culture and accumulation of cellular debris in the culture suggested that the cells lysed as they attempted to grow and that lysis was the ultimate reason for the death of the *surA rpoS* double mutants in stationary phase.

Cell shape is determined by the murein sacculus, which is composed of short, cross-linked strands of peptidoglycan (14). The altered morphology of  $\Delta surA3::Km rpoS::Tn10$  cells suggested that changes in peptidoglycan composition occurred in these mutant cells. At least 15 enzymes act in concert to synthesize the sacculus (9, 14). Although disruption of any one of these enzymes need not be lethal to the cell, morphological defects can occur (14). The concurrent disruption of multiple peptidoglycan-synthesizing enzymes can cause cell lysis, as evidenced by the action of  $\beta$ -lactam antibiotics (38). The misshapen morphology of  $\Delta surA3::Km rpoS::Tn10$  cells suggested that the relative activities of these sacculus-synthesizing enzymes were no longer in balance, resulting in a sacculus with structural defects.

The fine structure of a sacculus can be determined by isolating the peptidoglycan from the cell and then digesting it into its component mucopeptides (8). At least 80 different mucopeptides are generated from this digestion, and these can be separated by reverse-phase HPLC (8). Changes in a chromatogram profile reflect changes in relative enzyme activities *in vivo*. This technique has been used extensively to monitor the changes that a sacculus undergoes under a variety of conditions, including the changes seen upon the entry of cells into stationary phase (27, 30, 44). The peptidoglycans of the wild type and the *surA1::mini-Tn10(Km) rpoS819* mutant were analyzed by this technique. The results indicated that the mutant cells had an approximately twofold enrichment of mucopeptides with 1,6-anhydrous moieties. These moieties are the signature activities of the lytic transglycosylases, enzymes that break the  $\beta$ -1,4-acetylglucosaminic links between *N*-acetyl-muramic acid and *N*-acetyl-glucosamine residues of the peptidoglycan backbone (15). This activity is analogous to that of lysozyme, so it is not surprising that increasing the amount of it can result in cell lysis.

At least three lytic transglycosylases are present in *E. coli*,

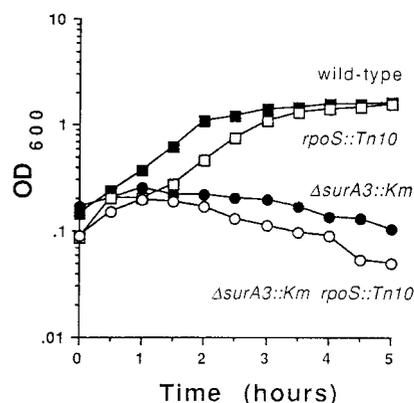


FIG. 3. SurA is required for growth at pH 9. Exponentially growing cells ( $OD_{600}$ , 0.1) were resuspended into LB medium (pH 9) and incubated for 5 h at 37°C with aeration. Growth was monitored by observing OD. Shown are results for the wild type (filled squares), the *rpoS::Tn10* mutant (open squares), the  $\Delta surA3::Km$  mutant (filled circles), and the *rpoS::Tn10*  $\Delta surA3::Km$  double mutant (open circles). This experiment was repeated more than three times, and representative data are shown.

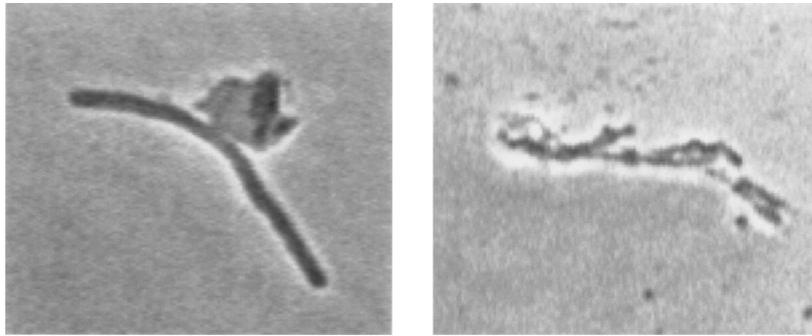


FIG. 4. Typical morphology of *surA1::mini-Tn10(Km) rpoS819* cells in the process of lysing after transfer to pH 9. Cultures of *surA1::mini-Tn10(Km) rpoS819* cells growing exponentially ( $OD_{600}$  0.1) in LB medium were transferred to LB medium at pH 9. After 30 min at 37°C cells were observed microscopically with phase contrast.

the predominant one being the 70-kDa soluble lytic transglycosylase (Slt70) (35). These three enzymes are required for cellular growth, since links within a sacculus must be broken in order for new strands of peptidoglycan to be inserted. Little is known about the regulation of any of the peptidoglycan-metabolizing enzymes. For instance, overexpression of Slt70 is insufficient to lyse cells unless the stringent response is inactivated, but the mechanism(s) by which the stringent response is involved is still unknown (2). This lack of cell lysis under these conditions suggests that although the relative amount of lytic transglycosylase activity is probably increased in the *surA1::*

*mini-Tn10(Km) rpoS819* strain, some other activities must also be altered in order for lysis to occur.

To investigate what other cell wall-synthesizing activities might have been altered in the  $\Delta$ *surA3::Km rpoS::Tn10 double mutant, the relative amounts of nine PBPs were measured. These enzymes catalyze a variety of functions required for the growth of the sacculus. A penicillin-fluorescein conjugate, Flu-Apa, was used to visualize and quantitate the PBPs (32). Wild-type and  $\Delta$ *surA3::Km* cells were incubated with Flu-Apa, and the proteins were then separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 6). During growth at*

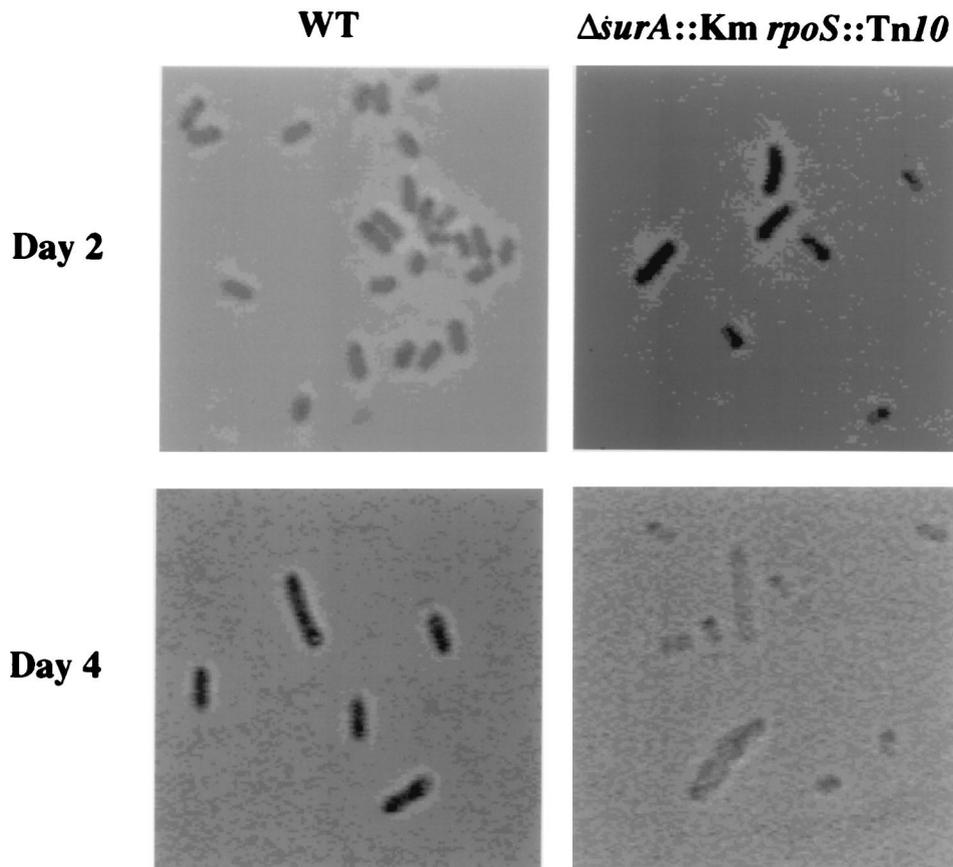


FIG. 5. Morphology of  $\Delta$ *surA3::Km rpoS::Tn10* cells in stationary phase. The morphologies of wild-type (WT) and  $\Delta$ *surA3::Km rpoS::Tn10* cells at days 2 and 4 of incubation in LB medium are shown.

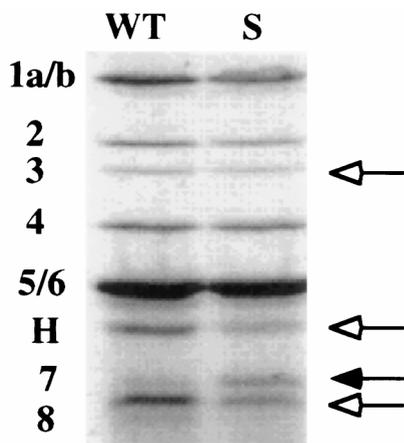


FIG. 6. The pattern of PBPs is altered in a  $\Delta surA3::Km$  mutant. Cells from exponential-phase cultures ( $OD_{600}$ , 0.1) grown in LB medium at 37°C were labeled with Flu-Apa (see the text). Shown are bands that decreased (open arrows) or increased (filled arrow) in intensity in the mutant relative to the intensities of corresponding bands in the wild type. WT, wild type; S,  $\Delta surA3::Km$  mutant; H, AmpH. Numbers indicate designations of PBPs.

neutral pH the amounts of several PBPs were altered in the  $\Delta surA3::Km$  mutant. Most notably, PBP8 and AmpH decreased and PBP7 increased. The amounts of PBP1, -2, -3, and -8 and AmpH decreased between 15 and 40% relative to amounts in the wild type, while the amount of PBP4 was approximately the same. In overnight cultures few differences were observed (data not shown). Since cultures of stationary-phase cells contain mixtures of viable and nonviable cells, the PBPs were not measured during extended incubation.

PBP7 is an endopeptidase that cleaves the DD-diaminopimelate-D-alanine bonds in intact cell walls, the same bond that PBP3 creates (33). PBP7 is processed into PBP8, a smaller but still active form (12, 33). AmpH is similar to the class C  $\beta$ -lactamases but does not possess  $\beta$ -lactamase activity (13). Cells lacking AmpH as well as PBP1a and PBP5 have aberrant morphology during exponential growth, though they have no noticeable defect when AmpH alone is missing (13).

It has been reported that PBP3, PBP7/8, and Slt70 might function together in a complex and that the activity of Slt70 might be modulated by the complex (34). PBP3, encoded by *ftsI*, forms the cross-links between mucopeptide side chains that create the septa of dividing cells (14). Inactivation of PBP3 does not cause cell lysis but rather inhibits cell division, leading to filament formation (14). Since the HPLC data suggested that Slt activity might be increased and since the PBP profile data indicated that the amounts of PBP7/8 were altered, we decided to test whether a mutation in one of the genes of this complex, *ftsI*, would confer a  $Sur^-$  phenotype upon the cell. To do this, we tested whether cells lacking  $\sigma^S$  and PBP3 lysed during stationary phase. Although loss of PBP3 results in filamentation in growing cells, the effect of losing this enzyme in stationary-phase cultures was unknown. PBP3 is essential for growth, so mutations in the gene encoding PBP3, *ftsI*, are conditional mutations. These cultures must be maintained at the permissive temperature, 30°C, and switched to 37°C to deplete PBP3 from the cells. The *ftsI23* mutation (11) was transduced into our wild-type strain, as well as into the isogenic *rpoS::Km* mutant. Cultures were grown at 30°C for 15 h, and then the temperature was shifted to 37°C for long-term incubation of the cultures in stationary phase (Fig. 7). Control cell cultures maintained at 30°C displayed normal stationary-phase survival characteristics, similar to what occurred with the wild

type (Fig. 7A). Cells in cultures whose temperature was shifted to 37°C behaved differently, mimicking the  $Sur^-$  phenotype. The *ftsI23 rpoS::Km* double mutant completely lost viability within 6 to 7 days, similar to what occurred with a *surA rpoS* mutant (Fig. 7B). No decrease in viability was seen in an *rpoS*<sup>+</sup> background, again similar to what occurred in a *surA rpoS*<sup>+</sup> background (Fig. 7B).

The morphology of the *ftsI23* mutants in stationary phase was also similar to that of *surA* mutants. After the switch to 37°C, in an otherwise wild-type background cells carrying the *ftsI23* allele appeared normal (Fig. 8). However, cells from overnight cultures of the double mutant appeared long and thin, reminiscent of the morphology of the  $\Delta surA3::Km rpoS::Tn10$  mutant (Fig. 8). As incubation continued and viability decreased, the cells bulged and lysed, which is again very similar to what occurred with the morphology of the  $\Delta surA3::Km rpoS::Tn10$  mutant (Fig. 8).

## DISCUSSION

We have investigated the requirement for SurA in stationary-phase cultures. SurA is expressed in both exponentially

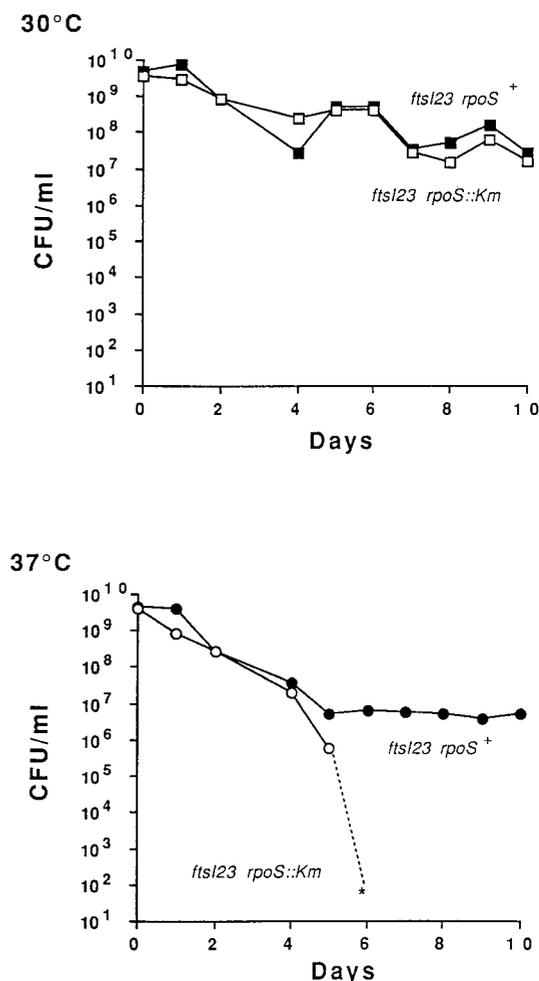


FIG. 7. Survival of *ftsI* mutants in stationary phase. *E. coli ftsI23* strains containing *rpoS* (filled symbols) or *rpoS::Km* (open symbols) were grown at 30°C. The cultures were either maintained at 30°C (top) or switched to 37°C (bottom) after 15 h of incubation. The asterisk represents  $<10^2$  CFU/ml. This experiment was repeated three times, and representative data are shown.

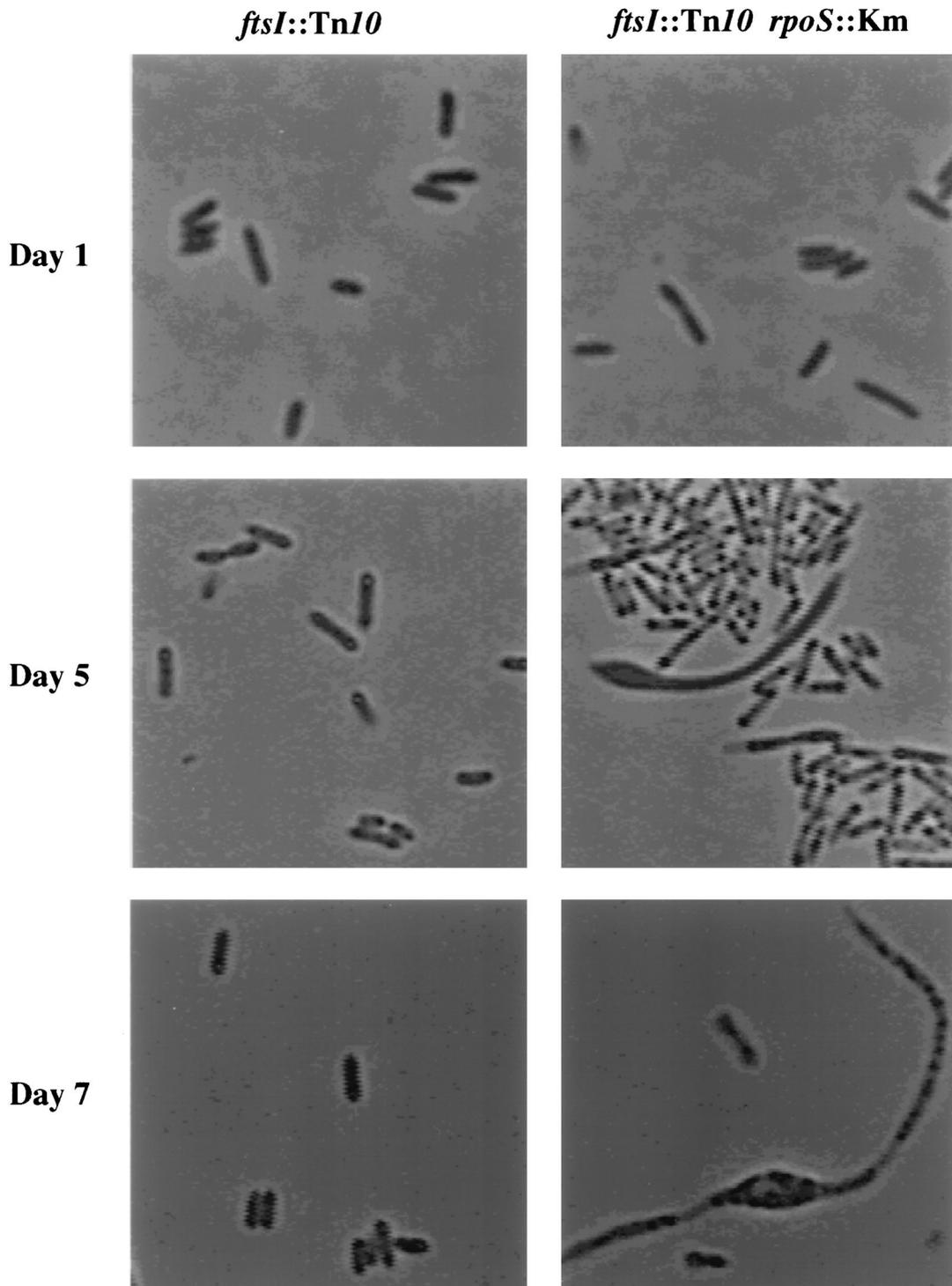


FIG. 8. Morphology of cells lacking *ftsI* in stationary phase. The morphologies of cells carrying *ftsI23* or *ftsI23 rpoS::Km* at days 1, 5, and 7 of incubation are shown. The cultures were grown at 30°C for 15 h and then switched to 37°C.

growing and nongrowing cells and is not regulated by  $\sigma^S$ . In the absence of SurA, some outer membrane proteins do not fold properly (22), resulting in pleiotropic defects, including increased sensitivity to antibiotics and large hydrophobic compounds (21). These phenotypes are observed in both *rpoS*<sup>+</sup> and *rpoS* mutant cells. Cells lacking SurA grow in LB medium

under standard laboratory conditions without noticeable defects. However, cells growing at pH 9 require SurA, presumably to allow nascent outer membrane proteins to fold. The pH of LB medium stationary-phase cultures slowly increases to pH 9, causing the majority of cells in the culture to lose viability. Presumably the death of the majority of cells provides nutri-

ents for the GASP mutants to grow. For wild-type cells or cells lacking either SurA or  $\sigma^S$ , this is not a problem. However, loss of both of these proteins prevents the cell from growing at pH 9 by causing cell lysis.

The results presented here indicate that lytic transglycosylase activity is increased in the *surA1::mini-Tn10(Km) rpoS819* mutant relative to that in the wild type and that the levels of PBP7/8 and AmpH are altered in the  $\Delta$ *surA3::Km* mutant. Since Slt is believed to be in a complex with PBP7/8 and PBP3, we speculate that in cells lacking SurA this complex is altered, perhaps destabilized, resulting in a disruption of the regulatory interactions between the enzymes. Presumably at high pH this complex or some other enzyme involved in sacculus integrity is lost, resulting in lysis.

The reason for the altered levels of PBPs is unclear. Four enzymes, namely, Slt70, endopeptidases 30 and 49, and PBP3, were tested for the ability to fold in the absence of SurA at neutral pH. These proteins do not require SurA function to fold properly (22; data not shown). Presumably, some other protein which does require SurA to fold alters the stability of the PBP3–PBP7/8–Slt70 complex. Alternatively, these enzymes might require SurA function to fold only at elevated pH.

The changes in peptidoglycan and the PBPs caused by loss of SurA are all observed in exponentially growing cells, at a time when cellular growth and morphology appears normal (data not shown). The morphology and growth defects are observed only in cells growing at alkaline pH in the absence of  $\sigma^S$ . Several enzymes involved in cell shape and structure are regulated by  $\sigma^S$ , namely, *bolA*, *ftsQAZ*, and *dacA* (3, 7). Mutant cells lacking *rpoS* have been reported to have altered shape in overnight cultures (19). The combined loss of SurA and  $\sigma^S$  presumably results in the altered expression of numerous proteins required for maintaining the cell structure, and loss of these enzymes results in a compromised structure that fails to maintain cell integrity at elevated pH. The lack of  $\sigma^S$ -dependent functions that mediate extreme alkali resistance (41) might also contribute to cell lysis of the mutants in stationary phase. Alternatively,  $\sigma^S$  may regulate some other folding chaperone that can partially supplement

SurA function.

#### ACKNOWLEDGMENTS

We thank Elaine Tuomanen for guidance and interpretation of HPLC results, David Weiss for Flu-Apa, the Beckwith lab for strains, and K. Young for strains and sharing information prior to publication. We also thank S. Finkel for critical reading of the manuscript.

This work was supported by a grant from the National Science Foundation.

#### REFERENCES

- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1992. Short protocols in molecular biology. John Wiley and Sons, New York, N.Y.
- Betzner, A. S., L. C. S. Ferreira, J.-V. Höltje, and W. Keck. 1990. Control of the activity of the soluble lytic transglycosylase by the stringent response in *Escherichia coli*. FEMS Microbiol. Lett. 67:161–164.
- Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter. 1991. Stationary-phase-inducible “gearbox” promoters: differential effects of *katF* mutations and the role of  $\sigma^{70}$ . J. strain Bacteriol. 173:4482–4492.
- Connell, N., Z. Han, F. Moreno, and R. Kolter. 1987. An *E. coli* promoter induced by the cessation of growth. Mol. Microbiol. 1:195–201.
- Danese, P. N., and T. J. Silhavy. 1997. The  $\sigma^E$  and the Cpx signal transduction systems control the synthesis of periplasmic protein-folding enzymes in *Escherichia coli*. Genes Dev. 11:1183–1193.
- Danese, P. N., W. B. Snyder, C. L. Cosma, L. J. B. Davis, and T. J. Silhavy. 1995. The Cpx two-component signal transduction pathway of *Escherichia coli* regulates transcription of the gene specifying the stress-inducible periplasmic protease, DegP. Genes Dev. 9:387–398.
- Dougherty, T. J., and M. J. Pucci. 1994. Penicillin-binding proteins are regulated by *rpoS* during transitions in growth states of *Escherichia coli*. Antimicrob. Agents Chemother. 38:205–210.
- Glauner, B. 1988. Separation and quantification of mucopeptides with high performance liquid chromatography. Anal. Biochem. 172:451–464.
- Glauner, B., and J. V. Höltje. 1990. Growth pattern of the murein sacculus of *Escherichia coli*. J. Biol. Chem. 265:18988–18996.
- Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Hedge, P. J., and B. G. Spratt. 1985. Resistance to  $\beta$ -lactam antibiotics by remodeling the active site of an *Escherichia coli* penicillin-binding protein. Nature 318:478–480.
- Henderson, T. A., M. Templin, and K. D. Young. 1995. Identification and cloning of the gene encoding penicillin-binding protein 7 of *Escherichia coli*. J. Bacteriol. 177:2074–2079.
- Henderson, T. A., K. D. Young, S. A. Denome, and P. K. Elf. 1997. AmpC and AmpH, proteins related to the class C beta-lactamases, bind penicillin and contribute to the normal morphology of *Escherichia coli*. J. Bacteriol. 179:6112–6121.
- Höltje, J.-V. 1995. From growth to autolysis: the murein hydrolases in *Escherichia coli*. Arch. Microbiol. 164:243–254.
- Höltje, J.-V., D. Mirelman, N. Sharon, and U. Schwarz. 1975. Novel type of murein transglycosylase in *Escherichia coli*. J. Bacteriol. 24:1067–1076.
- Horne, S. M., and K. D. Young. 1995. *Escherichia coli* and other species of the *Enterobacteriaceae* encode a protein similar to the family of Mip-like FK506-binding proteins. Arch. Microbiol. 163:357–365.
- Ivanova, A., M. Renshaw, R. V. Guntaka, and A. Eisenstark. 1992. DNA base sequence variability in *katF* (putative sigma factor) gene of *Escherichia coli*. Nucleic Acids Res. 20:5479–5480.
- Jishage, M., and A. Ishihama. 1997. Variation in RNA polymerase sigma subunit composition with different stocks of *Escherichia coli* W3110. J. Bacteriol. 179:959–963.
- Lange, R., and R. Hengge-Aronis. 1991. Growth phase-regulated expression of *bolA* and morphology of stationary-phase *Escherichia coli* cells are controlled by the novel sigma factor  $\sigma^S$  (*rpoS*). J. Bacteriol. 173:4474–4481.
- Lange, R., and R. Hengge-Aronis. 1991. Identification of a central regulator of stationary phase gene expression in *E. coli*. Mol. Microbiol. 5:49–59.
- Lazar, S. W. 1996. Survival of *Escherichia coli* in stationary phase. Ph.D. thesis. Harvard University, Cambridge, Mass.
- Lazar, S. W., and R. Kolter. 1996. SurA assists the folding of *Escherichia coli* outer membrane proteins. J. Bacteriol. 178:1770–1773.
- Liu, J., and C. T. Walsh. 1990. Peptidyl-prolyl *cis-trans*-isomerase from *Escherichia coli*: a periplasmic homolog of cyclophilin that is not inhibited by cyclosporin A. Proc. Natl. Acad. Sci. USA 87:4028–4032.
- Loewen, P. C. 1984. Isolation of catalase-deficient *Escherichia coli* mutants and genetic mapping of *katE*, a locus that affects catalase activity. J. Bacteriol. 157:622–626.
- Losick, R., and L. Shapiro. 1984. Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Matin, A. 1991. The molecular basis of carbon-starvation-induced general resistance in *Escherichia coli*. Mol. Microbiol. 5:3–10.
- Mengin-Lecreux, D., and J. Van Heijenoort. 1985. Effect of growth conditions on peptidoglycan content and cytoplasmic steps of its biosynthesis in *Escherichia coli*. J. Bacteriol. 163:208–212.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- Missiakas, D., J.-M. Betton, and S. Raina. 1996. New components of protein folding in the extracytoplasmic compartments of *Escherichia coli* SurA, FkpA and Skp/OmpH. EMBO J. 21:871–884.
- Pisabarro, A. G., M. A. de Pedro, and D. Vazquez. 1985. Structural modifications in the peptidoglycan of *Escherichia coli* associated with changes in the state of growth of the culture. J. Bacteriol. 161:238–242.
- Pogliano, J., A. S. Lynch, D. Belin, E. C. C. Lin, and J. Beckwith. 1997. Regulation of *Escherichia coli* cell envelope proteins involved in protein folding and degradation by the Cpx two-component system. Genes Dev. 11:1169–1182.
- Popham, D. L., and P. Setlow. 1996. Phenotypes of *Bacillus subtilis* mutants lacking multiple class A high molecular weight penicillin-binding-proteins. J. Bacteriol. 178:2079–2085.
- Romeis, T., and J. V. Höltje. 1994. Penicillin-binding protein 7/8 of *Escherichia coli* is a DD-endopeptidase. Eur. J. Biochem. 224:597–604.
- Romeis, T., and J. V. Höltje. 1994. Specific interaction of penicillin-binding proteins 3 and 7/8 with soluble lytic transglycosylase in *Escherichia coli*. J. Biol. Chem. 269:21603–21607.
- Romeis, T., W. Vollmer, and J.-V. Höltje. 1993. Characterization of three different lytic transglycosylases in *Escherichia coli*. FEMS Microbiol. Lett. 111:141–146.
- Rouviere, P. E., and C. A. Gross. 1996. SurA, a periplasmic protein with peptidyl-prolyl isomerase activity, participates in the assembly of outer membrane porins. Genes Dev. 10:3170–3182.
- Rudd, K. E., H. J. Sofia, E. V. Koonin, G. Plunkett III, S. Lazar, and P. E. Rouviere. 1995. A new family of peptidyl-prolyl isomerases. Trends Biochem. Sci. 20:12–14.

38. **Satta, G., G. Cornaglia, A. Mazzariol, G. Golini, S. Valisena, and R. Fontana.** 1995. Target for bacteriostatic and bacteriocidal activities of beta-lactam antibiotics against *Escherichia coli* resides in different penicillin-binding proteins. *Antimicrob. Agents Chemother.* **39**:812–818.
39. **Schmid, F. X.** 1995. Prolyl-isomerases join the fold. *Curr. Biol.* **5**:993–994.
40. **Shapiro, L., D. Kaiser, and R. Losick.** 1993. Development and behavior in bacteria. *Cell* **73**:835–836.
41. **Small, P., D. Blankerhorn, D. Welty, E. Zinser, and J. L. Slonczewski.** 1994. Acid and base resistance in *Escherichia coli* and *Shigella flexneri*: role of *rpoS* and growth pH. *J. Bacteriol.* **176**:1729–1737.
42. **Smith, I., R. A. Slepecky, and P. Setlow.** 1989. Regulation of prokaryotic development. Structural and functional analysis of bacterial sporulation and germination. American Society for Microbiology, Washington, D.C.
43. **Tormo, A., M. Almirón, and R. Kolter.** 1990. *surA*, an *Escherichia coli* gene essential for survival in stationary phase. *J. Bacteriol.* **172**:4339–4347.
44. **Tuomanen, E., and R. Cozens.** 1987. Changes in peptidoglycan composition and penicillin-binding proteins in slowly growing *Escherichia coli*. *J. Bacteriol.* **169**:5308–5310.
45. **Zambrano, M. M., and R. Kolter.** 1993. *Escherichia coli* mutants lacking NADH dehydrogenase I have a competitive disadvantage in stationary phase. *J. Bacteriol.* **175**:5642–5647.
46. **Zambrano, M. M., and R. Kolter.** 1996. GASping for life in stationary phase. *Cell* **86**:181–184.
47. **Zambrano, M. M., D. A. Siegle, M. Almirón, A. Tormo, and R. Kolter.** 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science* **259**:1757–1760.