INFECTION AND IMMUNITY, May 1995, p. 1840–1847 Vol. 63, No. 5
0019-9567/95/$04.00+0

The *rpoS* Gene from *Yersinia enterocolitica* and Its Influence on Expression of Virulence Factors

MAITE IRIARTE, ISABELLE STAINIER, AND GUY R. CORNELIS*

Microbial Pathogenesis Unit, International Institute of Cellular and Molecular Pathology and Faculté de Médecine, Université Catholique de Louvain, B-1200 Brussels, Belgium

Received 20 October 1994/Returned for modification 2 January 1995/Accepted 3 February 1995

The chromosome of *Yersinia enterocolitica* encodes a heat-stable enterotoxin called Yst and a surface antigen called Myf, which closely resembles enterotoxin-associated fimbriae. Both factors could act in conjunction to produce diarrhea. Production of the enterotoxin is regulated by temperature, osmolarity, and pH and occurs only when bacteria reach the stationary phase. Myf production is regulated by temperature and pH and, as we show in this work, also occurs after the exponential growth phase. In an attempt to understand the late-phase expression of *yst* and *myf*, we cloned, sequenced, and mutagenized the gene encoding RpoS, an alternative sigma factor of the RNA polymerase involved in expression of stationary-phase genes in other enterobacteria. An intact *rpoS* gene was necessary for full expression of *yst* in the stationary phase but not for the expression of *myf* and of pYV-encoded virulence determinants.

Yersinia enterocolitica is an enterobacterium responsible for gastroenteric syndromes (5, 11). Full virulence expression requires a 70-kb plasmid responsible for resistance to nonspecific host defenses (8). At 37°C and in the absence of Ca^{2+}, the pYV plasmid directs secretion of at least 11 proteins called Yops and an outer membrane protein called YadA. The chromosome of *Y. enterocolitica* encodes a heat-stable enterotoxin called Yst (12, 13, 47) and a surface antigen called Myf, which closely resembles enterotoxin-associated fimbriae described in other bacteria (24). Myf could be the colonization factor acting in conjunction with Yst to cause diarrhea, but this has not yet been demonstrated. Secretion of the enterotoxin is regulated by several environmental parameters, including temperature, osmolarity, and pH (4, 42). It is also strongly growth phase dependent: it occurs only when bacteria reach the stationary growth phase. Myf production is also known to be regulated by temperature and pH (23), but the influence of growth phase has not been studied to date. In the present paper, we show that *myf* genes, like *yst*, are late-phase genes. In *Escherichia coli* and *Salmonella typhimurium*, at least 40 genes are expressed specifically during the stationary phase (for reviews, see references 18, 29, and 54). The intracellular signals that lead to this expression have not yet been completely identified, but the product of the *rpoS* gene (33, 40), an alternative sigma subunit of RNA polymerase (58), is involved in the expression of some of these late genes (1, 2, 6, 7, 14, 19, 20, 25, 27, 32, 34, 37, 45, 50, 59, 61, 62). The *rpoS* gene of *S. typhimurium* controls expression of the *spv* genes (30), is required for virulence in mice (15, 30), and regulates starvation survival genes (46), while the *rpoS* gene from *Shigella flexneri* is required for development of the acid resistance which allows bacteria to survive passage through the stomach (55). The growth phase dependence of *yst* and *myf* expression in *Y. enterocolitica* suggests that these genes may also require a homolog of RpoS. In an attempt to understand the mechanism of late-phase expression of *yst* and *myf*, we have cloned, sequenced, and mutagenized the *rpoS* homolog of *Y. enterocolitica*. We show that an intact *rpoS* gene is necessary for normal expression of *yst* in the stationary phase.

* Corresponding author. Mailing address: Microbial Pathogenesis Unit, Avenue Hippocrate 74, UCL 74.49, B-1200 Brussels, Belgium. Phone: 32 2 764 74 49. Fax: 32 2 764 74 98. Electronic mail address: cornelis@mipa.ucl.ac.be.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. *Y. enterocolitica* W1024 is a serotype O:9 strain. SI1024 is a *rpoS*::*aphA-3* mutant described in this paper. *E. coli* LK111, *E. coli* XL1-Blue (Stratagene, La Jolla, Calif.), and *E. coli* S-17-1 were used for standard genetic manipulations. *S. typhimurium* C53 (wild type) and C53K (*katF*::*kan*) (30) were kindly provided by F. Norel (Institut Pasteur, Paris, France). The plasmids used are listed in Table 1.

Bacteria were grown routinely on Trypticase soy broth (TSB; Oxoid, Hampshire, England) containing 0.3% (wt/vol) yeast extract and on Trypticase soy agar (TSA; Diagnostic Pasteur, Marnes la Coquette, France). For the analysis of *myf* expression, *Y. enterocolitica* was grown on brain heart infusion broth (Difco, Detroit, Mich.) supplemented with 0.5% yeast extract (Gibco BRL, Paisley, United Kingdom), 2.5 mM $CaCl_2$, and 0.2% xylose (SBHI) and adjusted to different pH values as described by Iriarte and Cornelis (23). For the induction of the *yop* regulon, *Y. enterocolitica* was grown in brain heart infusion broth (Difco) supplemented with 4 mg of glucose ml^{-1}, 20 mM $MgCl_2$, and 20 mM Na oxalate. The antibiotics used for selection procedures were nalidixic acid (35 $\mu g \cdot ml^{-1}$), ampicillin (200 $\mu g \cdot ml^{-1}$), streptomycin (100 $\mu g \cdot ml^{-1}$), kanamycin (50 $\mu g \cdot ml^{-1}$), and tetracycline (10 $\mu g \cdot ml^{-1}$).

DNA and RNA manipulations. RNA extraction and analysis were done as described by Lambert de Rouvroit et al. (31). The probe used to detect the *myfA* transcript was prepared as described previously (24).

The DNA sequence of *rpoS* and flanking regions was determined by the method of Sanger et al. (51) with plasmids pSI6, pSI9, and pSI12. DNA and protein sequences were analyzed with the Fasta program (48). The isoelectric point was calculated with the GCG (Genetics Computer Group sequence analysis software package [University of Wisconsin, Madison]) computer program.

Cloning of the *rpoS* gene from *Y. enterocolitica*. We pooled the 760 clones of our W1024 genomic library in pLAFR3 (13) in groups of 10 clones and extracted cosmid DNA. DNA was digested by *Eco*RI and *Hin*dIII, separated by gel electrophoresis, transferred to membranes, and hybridized with a 1.3-kb *Bam*HI-*Hin*dIII fragment of plasmid pPS1.3 containing *rpoS* from *S. flexneri* (55). Cosmids pIDD84, pIDE9, pIDD37, pIDG88, and pIDI59 hybridized with the probe, but further Southern blot analysis did not detect any restriction fragment common to all of these cosmids, suggesting that some of them could be chimeric. When comparing the restriction patterns of the cosmids with that of chromosomal DNA from *Y. enterocolitica*, we identified a 3.5-kb *Eco*RV fragment that seemed to be common to cosmid pIDE9 and chromosomal DNA. Cosmid pIDE9 was thus chosen for further characterization.

We first subcloned the *Eco*RV fragment of pIDE9 into the corresponding site of pBlueScript KS^-, giving pSI5. The position of the *rpoS* homolog in the *Eco*RV fragment was determined by Southern blot analysis using the *Shigella rpoS* gene. A 1-kb *Eco*RI fragment of pSI5 hybridizing with this probe was subcloned in pBlueScript KS^-, giving pSI6. Unfortunately, sequence analysis showed that neither pSI6 nor pIDE9 contained the entire *rpoS* gene. They contained 348 bp of the upstream region of *rpoS* and the 5′ first 574 bp of *rpoS*. The *rpoS* gene was interrupted by a *Sau*3A-*Sma*I-*Eco*RI recognition sequence which corresponds to the polysite of cosmid pLAFR3, which was used to construct the genomic library.

TABLE 1. Plasmids used in this study

Plasmid	Characteristic(s)	Reference
pPS1.3	pACYC184 plus *Eco*RV-*Hin*dIII fragment containing *S. flexneri rpoS*	55
pIDE9	Member of the W1024 genomic library in pLAFR3	13
pSI5	pBlueScript KS$^-$ plus 3.5-kb *Eco*RV fragment of pIDE9	This work
pSI6	pBlueScript KS$^-$ plus 920-bp *Eco*RI fragment of pSI5; contains 570 bp of *rpoS* from *Y. enterocolitica*	This work
pSI7	pSI6 plus *aphA-3* cassette cloned in *Mun*I site of *rpoS* gene	This work
pSI8	pKNG101 plus 1.9-kb *Sal*I-*Xba*I fragment of pSI7; contains *rpoS′-aphA3-rpoS″*-disrupted gene	This work
pSI9	pBlueScript KS$^-$ plus 3.5-kb *Eco*RV fragment of *Y. enterocolitica* chromosomal DNA; contains complete *rpoS* gene	This work
pSI10	pPW62 plus 3.5-kb *Eco*RI-*Hin*dIII fragment of pSI9	This work
pSI11	pBlueScript SK$^-$ plus 3.5-kb *Eco*RI-*Hin*dIII fragment of pSI9	This work
pSI12	pBlueScript SK$^-$ plus 1.5-kb *Bam*HI-*Hin*dIII fragment of pSI9	This work
pKNG101	*pir-ori*$_{R6K}$ *oriT*$_{RK2}$ *strAB sacBR*	28
pUC18K	850-bp *aphA-3* cassette inserted into the *Sma*I site of plasmid pUC18	41
pSTF9	Transcriptional fusion *spvRAB′-cat* in pVK100	30
pID210	pID207 plus 1.2-kb *Bgl*II-*Hin*dIII fragment of pID6	42

Since *rpoS* was localized on a 3.5-kb *Eco*RV fragment of the chromosome, we digested total DNA from *Y. enterocolitica* W1024 with *Eco*RV and subcloned the fragments in the size range of 2.5 to 4 kb into the corresponding site of pBlueScript KS$^-$. The recombinant fragments were screened with the 1-kb *Eco*RI *Y. enterocolitica rpoS*-specific probe isolated from pSI6. Clone pSI9 contained the 3.5-kb *Eco*RV fragment enclosing the complete *rpoS* gene. A 1.5-kb *Eco*RV-*Bam*HI fragment of pSI9 was sequenced in both directions and found to contain the entire *rpoS* gene.

Nucleotide sequence accession number. The sequence of *rpoS* and flanking regions has been submitted to the GenBank nucleotide sequence data libraries under accession number U16152.

Construction of a *Y. enterocolitica* W1024 *rpoS* mutant. In a first step, plasmid pSI6 containing the fragment with 574 bp of the truncated *rpoS* gene was digested with *Mun*I, and the ends were filled-in with Klenow fragment. The cleavage site is located within the insert 461 bp from the ATG start codon of *rpoS*. In parallel, we extracted the *aphA-3* cassette (41) from its carrying plasmid, pUC18K, with enzymes *Eco*RI and *Bam*HI. Both ends were filled in with Klenow fragment. Insertion of the cassette in the filled *Mun*I site of pSI6 gave pSI7. In pSI7, the *aphA-3* cassette is flanked by 461 nucleotides of *rpoS* in the 5′ direction and 113 nucleotides of *rpoS* in the 3′ direction.

In a second step, we transferred the *Sal*I-*Xba*I fragment of pSI7 containing the *rpoS′-aphA3-rpoS″* construct into the corresponding sites of the suicide vector pKNG101 to obtain pSI8. pKNG101 contains a streptomycin resistance gene and the *sacB* gene encoding levansucrase and conferring sensitivity to sucrose (28). Thereafter, the mutator plasmid pSI8 was mobilized in *Y. enterocolitica* W1024. The chromosomal *rpoS* gene of strain W1024 was replaced by the *rpoS′-aphA3-rpoS″* construct by double recombination. The selection for the *rpoS* mutant was based on the acquired resistance to kanamycin, streptomycin sensitivity, and sucrose resistance. Gene disruption was finally checked by Southern blot hybridization. Chromosomal DNA from the wild type and the *rpoS* mutant was digested with *Eco*RV, transferred to nylon membranes, and hybridized with an *Eco*RI fragment of pSI6 containing 348 bp of the upstream region of *rpoS* and 574 bp of the *rpoS* gene. In the wild type, the probe hybridized with the original 3.5-kb *Eco*RV fragment, while in the *rpoS* mutant, it hybridized with a 1.5-kb *Eco*RV fragment generated by the insertion of the cassette (Fig. 1). The W1024 *rpoS* mutant was called SI1024.

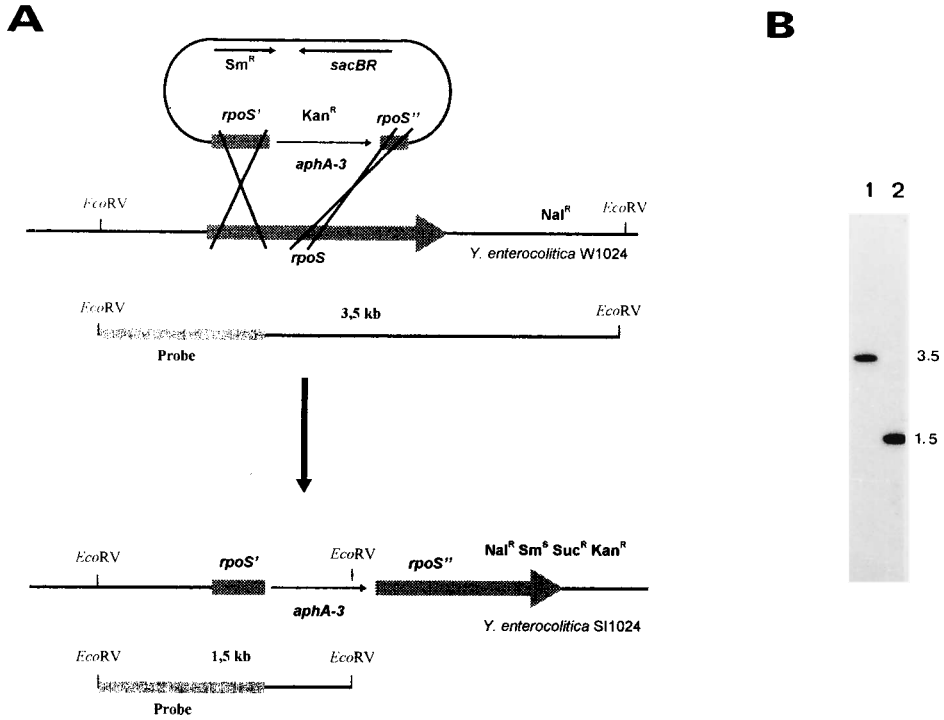


FIG. 1. (A) Generation of the *rpoS* mutant by double recombination with plasmid pSI8. (B) Southern hybridization analysis of the wild-type strain W1024 (lane 1) and the *rpoS* mutant SI1024 (lane 2). Chromosomal DNA was digested by *Eco*RV. The sizes of the fragments are given in kilobase pairs to the right of panel B.

Protein analysis. The Yops were prepared and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis as described by Cornelis et al. (10). β-Galactosidase was assayed as described by Cornelis et al. (9). Chloramphenicol acetyltransferase activity was determined by the method of Shaw (53). The *rpoS* gene product was identified with the bacteriophage T7 promoter-polymerase system of Tabor and Richardson (57) with *E. coli* LK111(pGP1-2)(pSI11).

Survival assays. Evaluation of prolonged survival was performed in TSB at 22°C for 5 weeks. Aliquots of bacteria were removed at different times, diluted, and plated directly on TSA supplemented with nalidixic acid (wild-type strain) or nalidixic acid and kanamycin (*rpoS* mutant).

The heat shock survival assay was performed mainly as described by Lange and Hengge-Aronis (33). Briefly, cells grown to the stationary phase were resuspended in 0.7% NaCl to a final concentration of about 5,000 cells · ml^{-1}. Two-milliliter samples were transferred to prewarmed flasks (52°C), and in 1-min intervals, 100-μl aliquots were plated directly on TSA supplemented with nalidixic acid or nalidixic acid and kanamycin.

Virulence assay. The 50% lethal dose (LD_{50}) was determined for groups of four 6-week-old female BALB/c mice by intraperitoneal administration of 2.5 mg of desferrioxamine (Desferal; CIBA-GEIGY, Brussels, Belgium) and 10^3 to 10^8 bacteria.

To study invasion, *Y. enterocolitica* W1024 (wild type) and SI1024 (*rpoS* mutant) were inoculated in 8-week-old female BALB/c mice. Three groups of four mice were inoculated by gastric intubation with 300 μl of a suspension containing either 6×10^9 W1024 bacteria or 9×10^9 SI1024 bacteria and 2.5 mg of desferrioxamine. The mice were killed at 18 h, 2 days, and 7 days postinfection. For each mouse, three Peyer's patches were excised from the ileum, washed in 0.7% NaCl–0.1% Triton X-100, and homogenized in 5 ml of phosphate-buffered saline (PBS; 50 mM sodium phosphate, 150 mM NaCl [pH 7.4]) as described by Sory et al. (56). Appropriate dilutions of the homogenized tissues were plated immediately on TSA containing nalidixic acid (wild type) or nalidixic acid and kanamycin (*rpoS* mutant). The spleen was harvested from each mouse only at 2 and 7 days postinfection, resuspended in 5 ml of PBS, and homogenized, and the homogenate was plated as described for Peyer's patches.

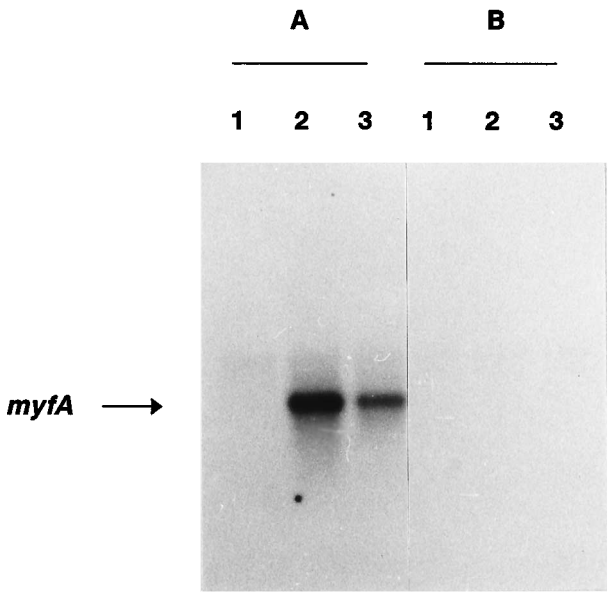


FIG. 2. Influence of growth phase and pH on transcription of *myfA*. Bacteria were grown at 37°C on SBHI adjusted to either pH 6 (A) or pH 8 (B). Total RNA was extracted at different time points throughout the bacterial growth curve. Lanes: 1, exponential phase; 2, transition between late exponential phase and early stationary phase; 3, after 2 h in stationary phase.

RESULTS

Expression of Myf is growth phase and pH dependent. To analyze the influence of growth phase on Myf expression, we grew *Y. enterocolitica* W1024 in SBHI adjusted to pH 6 and isolated the total RNA at different time points throughout the bacterial growth phase. We carried out a Northern (RNA) blot analysis with a *myfA* probe consisting of a central 280-bp part of the gene. As can be seen in Fig. 2, the *myfA* transcript was detectable only after bacteria had entered the stationary phase. It was more abundant in the transition from the late exponential phase to the early stationary phase than it was in the late stationary phase.

To study the relationship between growth phase and pH dependence, we grew bacteria in parallel at pH 6.0 and 8.0 and isolated RNA from bacteria in the exponential growth phase, in the transition between the exponential and stationary phases, and after at least 2 h in the stationary phase. As can be seen in Fig. 2, *myfA* was never transcribed when bacteria were in alkaline medium.

We repeated the same experiment with bacteria grown in parallel in SBHI (pH 6) at 37 and 22°C. Gene *myfA* was never transcribed when bacteria were grown at 22°C (data not shown). Thus, the three conditions (i.e., low pH, 37°C, and late phase) are always required for *myf* expression.

Cloning and sequencing of the *rpoS* gene from *Y. enterocolitica*. We wondered whether a homolog of RpoS might be involved in the expression of *myf* and *yst*. We thus decided to clone the putative *rpoS* allele of *Y. enterocolitica*.

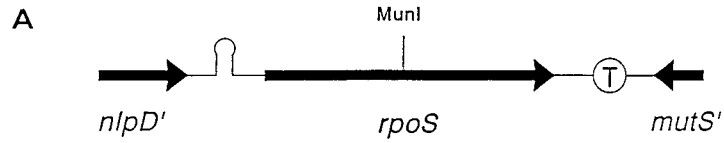
We probed our library of the *Y. enterocolitica* W1024 genome with the *rpoS* gene of *S. flexneri* (55). We then subcloned the relevant cosmid fragment in pBlueScript, giving pSI6. The sequence analysis revealed that pSI6 as well as the original cosmid contained only the 5′ end (574 bp) of a gene with a 90% homology to the *rpoS* gene of *E. coli*. This truncated *rpoS* DNA was then used as a probe to clone the complete *Y. enterocolitica* *rpoS* gene directly from chromosomal DNA (for details, see Materials and Methods).

The sequence of *rpoS* is presented in Fig. 3. The gene starts at a *Nde*I site at position 349 and ends at position 1342. It is preceded by a putative Shine-Dalgarno sequence (AGGAG), 6 bp upstream from the ATG start codon, and followed by a 30-bp palindromic sequence ending in a polyT 30 bp downstream from the TAA stop codon. The *rpoS* gene encodes a protein of 331 amino acids with a predicted molecular mass of 38 kDa and a theoretical isoelectric point of 4.57. The comparison of the predicted amino acid sequence with the PIR and GenBank sequence databases indicated 90% identity with sequences reported for RpoS in *E. coli* (38, 43), *S. typhimurium* (30), and *S. flexneri* (55). The identity with the corresponding protein in *Pseudomonas aeruginosa* (accession number D26134) was 74.5%. As expected, RpoS from *Y. enterocolitica* was also related to other σ factors, including the vegetative σ^{70} factor of *E. coli*, but the degree of similarity with these σ factors was significantly lower than that with the sequences mentioned above.

Upstream from *rpoS*, the coding region for the carboxy-terminal end (97 amino acids) of a putative protein which displays 90% identity to the NlpD protein recently described in *E. coli* (22, 35) was identified (Fig. 3). In *Y. enterocolitica*, the stop codon of the *nlpD* gene is separated by 54 bp from the start codon of the *rpoS* gene. This intercistronic region contains a palindromic sequence of 19 bp, but no promoter consensus sequence could be identified immediately upstream from the *rpoS* gene. Genes *nlpD* and *rpoS* have the same transcription polarity.

Downstream from *rpoS*, and in the opposite orientation, the coding region for the carboxy-terminal end (34 amino acids) of a protein which displays 85% identity to the carboxy-terminal end of the MutS protein of *E. coli* and *S. typhimurium* (17, 52) was identified (Fig. 3). The stop codon of *mutS* in *Y. enterocolitica* is separated by 28 bp from the terminator of *rpoS*.

It is worth noting that the *nlpD*, *rpoS*, and *mutS* genes are located in *Y. enterocolitica* in the same relative positions and



B

```
v        v        v        v        v        v        v
  GATATCGCCGGTTCTCGTGGGCAACCAATTTTCGCTACAGCAAATGGGCGAGTTGTGTATGCCGGGAA      68
 v        v        v        v        v        v        v
CGCACTTGGCGGTTACGGTAATCTAATCATCATCAAACACAATGATGATTACCTGAGCGCCTACGTCCAT     138
 v        v        v        v        v        v        v
AACGATACAATGCTGGTCCGGGAACAAGAAGAAGTGAAGGCGGGTCAAAAAATAGCAACCATGGGTAGCA     208
 v        v        v        v        v        v        v
CCGGAACCAGTTCAGTAAGATTGCACTTTGAAATTCGTTACAAGGGGAAATCCGTAAACCCGCTGCGTTA     278
 v        v        v        v        v        v        S.D.
CCTTCCGCAGCGATAGATTGGGCAGAATACGCTGTATTCTGCTCGCCGGATTCACGGGTAGGAGCAGCAT     348
       → Stop nlpD
 v        v        v        v        v        v        v
ATGAGCCAAAATACGCTGAAAGTTAACGAGTTGCATGAAGATGCTGATTTTGATGAAAACAGTACGGAAA     418
M  S  Q  N  T  L  K  V  N  E  L  H  E  D  A  D  F  D  E  N  S  T  E  T      24
 v        v        v        v        v        v        v
CTGAAATTTTCGATGAAAAAGCATTAGTAGAAGATGAACCTACTGAAAGCGAGTTAGCAGAAGATGAGCT     488
  E  I  F  D  E  K  A  L  V  E  D  E  P  T  E  S  E  L  A  E  D  E  L      47
 v        v        v        v        v        v        v
GTTGGCGCAAGGTGTTACCAGCGGTGCTGATGCGACACAGCTCTATCTTGGTGAGATTGGTTATTCGCCG     558
 L  A  Q  G  V  T  S  G  A  D  A  T  Q  L  Y  L  G  E  I  G  Y  S  P      70
 v        v        v        v        v        v        v
TTGTTGACCGCAGAAGAAGAGGTTTATTTTGCCCGGCGTGCATTGCGCGGAGATGTGCCTTCACGTCGGC     628
L  L  T  A  E  E  E  V  Y  F  A  R  R  A  L  R  G  D  V  P  S  R  R  R      94
 v        v        v        v        v        v        v
GTATGATCGAAAGTAACTTGCGGTTGGTAGTGAAGATTGCTCGCCGTTACAGTAATCGCGGTTTAGCGCT     698
  M  I  E  S  N  L  R  L  V  V  K  I  A  R  R  Y  S  N  R  G  L  A  L      117
 v        v        v        v        v        v        v
GCTGGATTTGATTGAAGAGGGTAACCTCGGTCTTATCCGTGCAGTGGAAAAGTTTGACCCAGAACGCGGT     768
 L  D  L  I  E  E  G  N  L  G  L  I  R  A  V  E  K  F  D  P  E  R  G      140
 v        v        v        v        v        v        v
TTCCGTTTCTCCACTTATGCCACATGGTGGATACGCCAGACAATTGAACGGGCAATAATGAACCAAACCC     838
F  R  F  S  T  Y  A  T  W  W  I  R  Q  T  I  E  R  A  I  M  N  Q  T  R     164
 v        v        v        v        v        v        v
GTACCATCCGTCTGCCTATCCATATCGTTAAAGAATTAAACGTTTATTTGCGTACAGCGCGAGAACTTTC     908
  T  I  R  L  P  I  H  I  V  K  E  L  N  V  Y  L  R  T  A  R  E  L  S      187
 v        v        v        v        v        v        v
TCATAAATTAGATCATGAACCGAGCGCAGAAGAGATTGCAGAGCAACTCGATAAGCCAGTTGATGATGTG     978
 H  K  L  D  H  E  P  S  A  E  E  I  A  E  Q  L  D  K  P  V  D  D  V      210
 v        v        v        v        v        v        v
AGTCGTATGTTGCGCCTTAACGAACGTATCACTTCTGTTGATACACCTTTAGGCGGCGATTCAGAGAAAG    1048
S  R  M  L  R  L  N  E  R  I  T  S  V  D  T  P  L  G  G  D  S  E  K  A     234
 v        v        v        v        v        v        v
CCTTGTTAGATATTCTGTCTGACGAAAATGAAAACGGCCCAGAAGACACCACGCAAGATGACGATATGAA    1118
  L  L  D  I  L  S  D  E  N  E  N  G  P  E  D  T  T  Q  D  D  D  M  K      257
 v        v        v        v        v        v        v
ACAAAGTATCGTTAAATGGCTGTTCGAATTGAATGCAAAACAGCGCGAAGTTCTGGCCCGTCGTTTTGGT    1188
 Q  S  I  V  K  W  L  F  E  L  N  A  K  Q  R  E  V  L  A  R  R  F  G      280
 v        v        v        v        v        v        v
CTGTTAGGATATGAAGCTGCAACACTGGAAGATGTTGGCCGTGAAATTGGTTTGACACGTGAACGTGTGC    1258
L  L  G  Y  E  A  A  T  L  E  D  V  G  R  E  I  G  L  T  R  E  R  V  R     304
 v        v        v        v        v        v        v
GCCAGATTCAGGTTGAAGGGTTGCGTCGTTTGCGGGAAATTCTGCAAGCGCAGGGCCTGAGCATCGAAGC    1328
  Q  I  Q  V  E  G  L  R  R  L  R  E  I  L  Q  A  Q  G  L  S  I  E  A      327
 v        v        v        v        v
ATTGTTCCGCGAATAGCGATACTACCGCAAACAGTCTGGAAAACACAAAAACGGTGAACTGATGTTCACC    1398
 L  F  R  E  <       331                                  Terminator
 v        v        v        v        v        v        v
GTTTTTTTATGGCGACAATTAACCTTTAACTGGTTTACACCATGTTTTTCAGGCGATAAATCCACTCAAG    1468
                              Stop mutS ←
 v        v        v        v        v        v        v
CGCCTGACGTGGTGATAGGGAGTCTGGATCCAATGACTCTAAGGCTTCCACTGCTGGCGATACCTCTTCA    1538
```

FIG. 3. (A) Schematic representation showing the positions and relative orientations of *nlpD*, *rpoS*, and *mutS* in the chromosome of *Y. enterocolitica*; (B) nucleotide sequence of the chromosomal loci containing the coding region for the carboxy-terminal end of NlpD, the complete RpoS protein, and the carboxy-terminal end of MutS. The putative Shine-Dalgarno ribosome binding site for *rpoS* is designated S.D. The terminator of *rpoS* is underlined. The deduced amino acid sequence of RpoS is shown. The directions of transcription of *nlpD* and *mutS* are shown by arrows, and the stop codons of both genes are underlined twice. The MutS protein is encoded by the strand complementary to that shown in the figure.

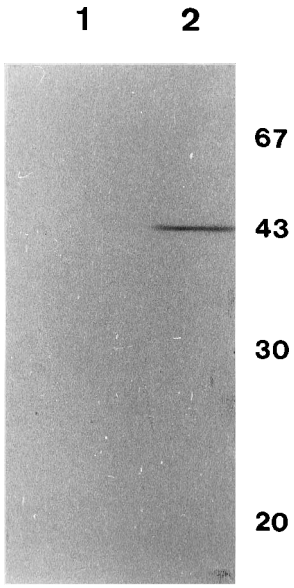


FIG. 4. Expression of the *rpoS* gene with the T7 RNA polymerase system. Lanes: 1, *E. coli* LK111(pGP1-2)(pBlueScript); 2, *E. coli* LK111(pGP1-2)(pSI11). The positions of the molecular mass markers (in kilodaltons) are indicated.

orientations as those of the corresponding genes in the *E. coli* chromosome (22, 35, 37).

Detection of RpoS. The analysis of the *rpoS* gene product by exclusive ^{35}S-methionine labelling with the T7 RNA polymerase system (57) revealed a protein with an apparent molecular mass of 43 kDa (Fig. 4). The discrepancy between this molecular mass and the theoretical one deduced from the nucleotide sequence (38 kDa) could be due to an influence of the very low isoelectric point of the protein on migration in SDS gels. The same discrepancy has also been observed for RpoS from *E. coli* by Tanaka et al. (58).

Construction of a *Y. enterocolitica rpoS* mutant. We constructed a *Y. enterocolitica rpoS* mutant by allelic exchange. To generate a nonpolar mutation in the *rpoS* gene, we used the *aphA-3* cassette described by Ménard et al. (41). This cassette contains a start codon in 3′ so that appropriate cloning will allow translation of the remaining 3′ portion of *rpoS* and eventual downstream genes. The details of the mutagenesis are given in Materials and Methods. Allelic exchange was checked by Southern hybridization (Fig. 1), and the engineered *Y. enterocolitica* (*rpoS*::*aphA-3*) mutant was called SI1024.

To further control that our strain SI1024 was phenotypically RpoS$^-$, we monitored the expression of a known RpoS-dependent gene. It has recently been shown that in *S. typhimurium*, *rpoS* regulates expression of *spv* genes (30). We introduced plasmid pSTF9 carrying a *spvRAB′-cat* transcriptional fusion (30) in *Y. enterocolitica* W1024(pYV$^-$) (wild type) and SI1024 (pYV$^-$) (*rpoS* mutant), and we measured the chloramphenicol acetyltransferase activity in extracts of bacteria grown to the stationary phase at 37°C. The values were compared with those obtained with extracts of *S. typhimurium* C53 (wild-type strain) and C53K (*rpoS* mutant) carrying the same plasmid (30). The chloramphenicol acetyltransferase activity was significantly lower in SI1024 extracts than in W1024 extracts (Table 2). These results confirm that the *rpoS* gene is not functional in our mutant strain SI1024.

TABLE 2. CAT activity of isogenic strains of *S. typhimurium* and *Y. enterocolitica* carrying a *spvRAB′-cat* fusion (30)

Strain	RpoS	CAT activity[a]
S. typhimurium C53(pSTF9)	+	1,296 ± 376
S. typhimurium C53K(pSTF9)	−	70 ± 6
Y. enterocolitica W1024(pSTF9)	+	643 ± 65
Y. enterocolitica SI1024(pSTF9)	−	24 ± 20

[a] Chloramphenicol acetyltransferase (CAT) activity was measured in extracts of bacteria grown to the stationary phase at 37°C in units per optical density at 600 nm.

Influence of the *rpoS* mutation on *yst* expression. To study the influence of the *rpoS* mutation on the expression of the *yst* enterotoxin gene, we used plasmid pID210, which contains a *yst′-lacZ* operon fusion (42). We introduced this plasmid in strains W1024(pYV$^-$) and SI1024(pYV$^-$) by conjugation and assayed β-galactosidase in bacteria grown to the stationary phase. The mean value for 5 colonies was 6461.6 ± 808.6 Miller units per optical density at 600 nm of W1024(pID210), while it was only 918 ± 946.8 Miller units per optical density at 600 nm of SI1024(pID210). Transcription of *yst* was thus significantly reduced but not completely abolished in the *rpoS* mutant.

To demonstrate that the *rpoS* mutation was responsible for the reduction of *yst* expression, we attempted to complement the mutation with a cloned *rpoS* gene. We subcloned a 3.5-kb *Eco*RI-*Hin*dIII fragment of pSI9 into pPW62, a high-copy-number mobilizable vector derived from pSelect. This construct, called pSI10, contains the 348-bp 5′ end of *nlpD* and the complete *rpoS* gene cloned downstream from the *lac* promoter. We introduced plasmid pSI10 by conjugation in *Y. enterocolitica* SI1024(pID210) and measured *yst-lacZ* transcription in bacteria grown to the stationary phase. Plasmid pSI10 did not restore β-galactosidase activity to wild-type levels, presumably because the cloned gene did not contain its own promoter.

Influence of *rpoS* mutation on the expression of the Myf antigen. To analyze the role of *rpoS* on transcription of *myf* genes, we extracted total RNA from *Y. enterocolitica* W1024 and SI1024 grown to the stationary phase at 37°C in acidic medium. We analyzed *myfA* RNA by Northern blotting and observed that expression of *myfA* was not significantly affected in the *rpoS* mutant (data not shown).

Influence of *rpoS* mutation in Yops and YadA production. We also tested the production of Yops and YadA by the *rpoS* mutant. We observed that there was no difference in the production of these proteins between the *rpoS* mutant and the wild-type strain (data not shown).

Virulence assay. We monitored the virulence for mice of *Y. enterocolitica* W1024 (*rpoS*$^+$) and of the *rpoS* mutant, SI1024, by determining the LD_{50} for mice inoculated intraperitoneally. The LD_{50} was approximately 10^6 for both strains. There was no significant difference.

To analyze invasion in vivo, we infected intragastrically two groups of 12 mice with either 6×10^9 W1024 bacteria or 9×10^9 SI1024 bacteria and determined the number of bacteria in Peyer's patches and spleens at 18 h, 2 days, and 7 days postinfection. The results, presented in Table 3, do not show any difference in the invasion capacities of the mutant and wild-type strains.

Survival assays. We measured the in vitro survival capacity of the *rpoS* mutant. After 5 weeks of culture in an aerated broth, the number of bacteria surviving was only fivefold lower for the *rpoS* mutant than for the wild type. The *rpoS* mutant showed, in addition, a slightly decreased ability to survive heat shock at 52°C (data not shown).

TABLE 3. Bacterial counts in Peyer's patches and spleens after intragastric inoculation of W1024 (*rpoS*$^+$) and SI1024 (*rpoS*) to mice

Strain	No. of bacteria recovered				
	Per Peyer's patch			Per spleen	
	18 h[a]	2 days	7 days	2 days	7 days
W1024	0.9×10^5	1.5×10^6	0.1×10^5	0.3×10^2	0
	1.2×10^5	2.2×10^6	0.5×10^5	0.03×10^2	0
	0.8×10^5	1.1×10^6	1.2×10^5	0.5×10^2	0
	4×10^5	1.6×10^6	0.8×10^5	50×10^2	0
Mean ± SD	$(1.7 \pm 1.5) \times 10^5$	$(1.6 \pm 0.5) \times 10^6$	$(0.6 \pm 05) \times 10^5$	$(12.7 \pm 23) \times 10^2$	
SI1024	2.2×10^5	1.6×10^6	2.4×10^5	12×10^2	1×10^2
	2.3×10^5	1.9×10^6	0.2×10^5	0.4×10^2	0
	1.7×10^5	1.9×10^6	1.9×10^5	17×10^2	0
	4×10^5	1.4×10^6	1.2×10^5	21×10^2	15×10^2
Mean ± SD	$(2.5 \pm 1) \times 10^5$	$(1.7 \pm 0.2) \times 10^6$	$(1.4 \pm 1) \times 10^5$	$(12.6 \pm 8.9) \times 10^2$	

[a] The Peyer's patches and spleens were excised after the indicated time. Four mice were sacrificed at each time point.

DISCUSSION

The chromosomal genes *yst* and *myf* are growth phase regulated, in the sense that none of them is expressed during exponential growth. The fact that both Yst and Myf are produced at the same stage argues in favor of a coordinate role for them in vivo. There are, however, minor differences in the expression profiles. Expression of *yst* begins when cells enter the late exponential to early stationary phase and continues throughout the stationary phase (42). In contrast, we observed here that *myfA* transcription occurs during the transition between the late exponential phase and the early stationary phase and then decreases gradually throughout the stationary phase.

Growth phase regulation is a highly complex phenomenon that is far from being completely understood. The expression of many stationary-phase genes in *E. coli* requires the alternative sigma factor RpoS (18, 33, 58). However, expression of many *rpoS*-dependent genes is significantly reduced but not completely abolished in a *rpoS* mutant (62), and many stationary-phase genes controlled by *rpoS* (*otsBA*, *treA*, *osmB*, and *osmY*) can also be induced during the exponential phase by increasing osmolarity (21, 32). Expression of *rpoS* itself is regulated by a complex mechanism at the levels of transcription, translation, and protein stability and is affected by external factors, including cyclic AMP (cAMP), ppGpp, cell density, and increase in osmolarity (16, 36, 38, 39, 44). These features indicate that RpoS is not the only factor involved in the regulation of stationary-phase genes. As the number of known late-phase genes increases, it becomes more evident that additional global regulatory proteins, including Lrp, the cAMP receptor protein (CRP)-cAMP complex, the integration host factor (IHF), and the histone-like protein (HNS), are also involved in the fine modulation of many *rpoS*-dependent stationary-phase genes. For example, the late-phase expression of *osmY* is regulated by the combined action of RpoS, Lrp, CRP-cAMP complex, and IHF acting independently from RpoS (32). The stationary-phase expression of Dps requires both RpoS and IHF (3); at least RpoS and the CRP-cAMP complex participate in the control of *glgS* (19), and late-phase expression of *csgA* results from the antagonist control of HNS and RpoS (45). In addition, growth-phase regulation of *treA*, *csi-12*, *csi-16*, and *csi-32* requires RpoS and additional regulatory mechanisms not yet understood (20, 62).

In this work, we characterized the homolog of *rpoS* in *Y. enterocolitica* to determine its role in the expression of virulence factors. As in the *E. coli* chromosome, *rpoS* appeared to be situated downstream from the *nlpD* gene and to be followed by the *mutS* gene. The RpoS protein of *Y. enterocolitica* has 90% identity with its *E. coli* counterpart. The carboxy-terminal end of the protein is 20 amino acids shorter than the *E. coli* sequence reported by Loewen et al. (38, 43) but is in agreement with the variations described by Ivanova et al. (26) for another *E. coli* strain.

As for virulence gene expression, RpoS seems to be involved, at least, in the growth phase regulation of *yst*. In a *rpoS* mutant, *yst* expression was reduced but not completely abolished. This suggests that, as for many *E. coli rpoS*-regulated genes, additional control mechanisms that also respond to growth phase are involved in the regulation of *yst*. Interestingly, *yst* expression is modulated by increasing osmolarity, and growth phase regulation of *yst* is also affected by the absence of the histone-like protein YmoA (42). The exact role of *rpoS* in *yst* regulation appears, therefore, to be quite complex. The upstream region of *yst* contains two major promoters with a -10 box similar to that of the σ^{70} promoter, but the -35 box is not evident (42). A promoter region with the same characteristics has also been identified in some *E. coli rpoS*-dependent genes (7, 32, 58, 63). In addition, Mikulskis et al. identified a putative gearbox sequence (60) upstream of *yst*, but deletion of this sequence had no effect on *yst* expression (42). Our present data do not allow us to conclude whether *rpoS* regulates *yst* directly by binding to the promoter region or indirectly through other regulatory proteins.

Plasmid pSI10 failed to complement the effect of *rpoS* on *yst* expression. This plasmid contains the entire *rpoS* gene, preceded by 348 bp encoding the carboxy-terminal end of NlpD. The intercistronic region between *rpoS* and *nlpD* does not seem to contain any promoter, which suggests that *nlpD* and *rpoS* form an operon. The same could also be suggested for *S. flexneri*: plasmid pPS1.3 containing the *rpoS* gene and 294 bp upstream of it does not fully restore the *rpoS*-dependent acid resistance of these bacteria. However, a larger plasmid probably containing *nlpD* and its promoter does complement (55). Lange and Hengge-Aronis have shown recently that, for *E. coli*, the promoter of *nlpD* is required for expression of *rpoS* (35). It is thus very likely that the lack of complementation by our cloned gene results from a transcription failure of the plasmid *rpoS* gene or from mRNA instability and not from a polar effect of the mutation on a downstream gene. The mutation that we generated in *rpoS* is indeed nonpolar because the *aphA-3* cassette contains a start codon in 3′ and the restriction sites used for its insertion in *rpoS* will allow translation of the remaining 3′ portion of the gene. Moreover, the gene

mutS situated immediately downstream of *rpoS* is oriented in the opposite direction to *rpoS*.

We showed that expression of *myfA* occurs at the onset of the stationary phase; however, it was not reduced significantly in the *rpoS* mutant. This type of situation is not unprecedented in *E. coli*: stationary-phase induction of both the *glgCAP* operon involved in glycogen synthesis and the *mcb* operon involved in microcin production is not controlled by *rpoS* (6, 19, 34). In conclusion, RpoS is required for *yst* expression but not for *myf* expression. Although the expression of these two virulence genes occurs in vitro at the same stage, it is regulated independently.

Pepe et al. (49) showed recently that expression of the invasin gene *inv* of *Y. enterocolitica* is also growth phase regulated. As for *yst*, the promoter region upstream from *inv* strongly resembles some *rpoS*-dependent promoters (49), leading the authors to suggest that *inv* expression could require RpoS.

We could not observe any difference between the LD_{50} of strain W1024 (*rpoS*$^+$) and that of strain SI1024 (*rpoS*::*aphA-3*) after intraperitoneal inoculation. This fits quite well with our observation that the *yop*, *ysc*, and *yadA* genes do not require RpoS. Our in vivo invasion assay also failed to reveal any difference between *rpoS*$^+$ and *rpoS* bacteria. This could be taken as an indication that the first steps of adhesion and invasion are RpoS independent. This observation requires some confirmation at the molecular level. Finally, the mouse model was inappropriate to test diarrhea, and the effect of the *rpoS* mutation on *yst* transcription would not affect the LD_{50}.

ACKNOWLEDGMENTS

We thank Vinh Ha Thi for technical assistance. We are grateful to P. Small (Rocky Mountain Laboratories, Hamilton, Mont.) for the gift of plasmid pPS1.3 and to F. Norel (Institut Pasteur) for the gift of plasmid pSTF9 and strains *S. typhimurium* C53 and C53K. We acknowledge R. Schulte and M.-P. Sory for discussion and for critically reading the manuscript. We also thank the I.C.P illustration service for the artwork.

This work was supported by the Belgian Fonds National de la Recherche Scientifique Medicale (convention 3.4627.93) and the Human Capital and Mobility Program from the European Community (contract ERBCHRXCT930171).

REFERENCES

1. **Aldea, M., T. Garrido, C. Hernández-Chico, M. Vicente, and S. R. Kushner.** 1989. Induction of a growth-phase-dependent promoter triggers transcription of *bolA*, an *Escherichia coli* morphogene. EMBO J. **8:**3923–3931.
2. **Almirón, M., A. J. Link, D. Furlong, and R. Kolter.** 1992. A novel DNA-binding protein with regulatory and protective roles in starved *Escherichia coli*. Genes Dev. **6:**2646–2654.
3. **Altuvia, S., M. Almirón, G. Huisman, R. Kolter, and G. Storz.** 1994. The *dps* promoter is activated by OxyR during growth and by IHF and σ^s in stationary phase. Mol. Microbiol. **13:**265–272.
4. **Amirmozafari, N., and D. C. Robertson.** 1993. Nutritional requirements for synthesis of heat-stable enterotoxin by *Yersinia enterocolitica*. Appl. Environ. Microbiol. **59:**3314–3320.
5. **Attwood, S. E. A., M. T. Cafferkey, and F. B. V. Keane.** 1989. *Yersinia* infections in surgical practice. Br. J. Surg. **76:**499–504.
6. **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 role of σ^{70}. J. Bacteriol. **173:**4482–4492.
7. **Chang, Y.-Y., A.-Y. Wang, and J. E. Cronan, Jr.** 1994. Expression of *Escherichia coli* pyruvate oxidase (PoxB) depends on the sigma factor encoded by the *rpoS*(*katF*) gene. Mol. Microbiol. **11:**1019–1028.
8. **Cornelis, G. R.** 1994. *Yersinia* pathogenicity factors. Curr. Top. Microbiol. Immunol. **192:**243–263.
9. **Cornelis, G. R., M.-P. Sory, Y. Laroche, and I. Derclaye.** 1986. Genetic analysis of the plasmid region controlling virulence in *Yersinia enterocolitica* O:9 by mini-mu insertions and *lac* gene fusions. Microb. Pathog. **1:**349–359.
10. **Cornelis, G. R., J.-C. Vanooteghem, and C. Sluiters.** 1987. Transcription of the *yop* regulon from *Y. enterocolitica* requires transacting pYV and chromosomal genes. Microb. Pathog. **2:**367–379.
11. **Cover, T. L., and R. C. Aber.** 1989. *Yersinia enterocolitica*. N. Engl. J. Med. **321:**16–24.
12. **Delor, I., and G. R. Cornelis.** 1992. Role of *Yersinia enterocolitica* Yst toxin in experimental infection of young rabbits. Infect. Immun. **60:**4269–4277.
13. **Delor, I., A. Kaeckenbeeck, G. Wauters, and G. R. Cornelis.** 1990. Nucleotide sequence of *yst*, the *Yersinia enterocolitica* gene encoding the heat-stable enterotoxin, and prevalence of the gene among pathogenic and nonpathogenic Yersiniae. Infect. Immun. **58:**2983–2988.
14. **Diaz-Guerra, L., F. Moreno, and J. L. San Millan.** 1989. *appR* gene product activates transcription of microcin C7 plasmid genes. J. Bacteriol. **171:**2906–2908.
15. **Fang, F. C., S. J. Libby, N. A. Buchmeier, P. C. Loewen, J. Switala, J. Harwood, and D. G. Guiney.** 1992. The alternative σ factor KatF (RpoS) regulates *Salmonella* virulence. Proc. Natl. Acad. Sci. USA **89:**11978–11982.
16. **Gentry, D. R., V. J. Hernandez, L. H. Nguyen, D. B. Jensen, and M. Cashel.** 1993. Synthesis of the stationary-phase sigma factor σ^s is positively regulated by ppGpp. J. Bacteriol. **175:**7982–7989.
17. **Haber, L. T., P. P. Pang, D. I. Sobell, J. A. Mankovich, and G. C. Walker.** 1988. Nucleotide sequence of the *Salmonella typhimurium mutS* gene required for mismatch repair: homology to MutS and HexA of *Streptococcus pneumoniae*. J. Bacteriol. **170:**197–202.
18. **Hengge-Aronis, R.** 1993. Survival of hunger and stress: the role of *rpoS* in early stationary phase gene regulation in *E. coli*. Cell **72:**165–168.
19. **Hengge-Aronis, R., and D. Fischer.** 1992. Identification and molecular analysis of *glgS*, a novel growth-phase-regulated and *rpoS*-dependent gene involved in glycogen synthesis in *Escherichia coli*. Mol. Microbiol. **6:**1877–1886.
20. **Hengge-Aronis, R., W. Klein, R. Lange, M. Rimmele, and W. Boos.** 1991. Trehalose synthesis genes are controlled by the putative sigma factor encoded by *rpoS* and are involved in stationary-phase thermotolerance in *Escherichia coli*. J. Bacteriol. **173:**7918–7924.
21. **Hengge-Aronis, R., R. Lange, N. Henneberg, and D. Fischer.** 1993. Osmotic regulation of *rpoS*-dependent genes in *Escherichia coli*. J. Bacteriol. **175:**259–265.
22. **Ichikawa, J. K., C. Li, J. Fu, and S. Clarke.** 1994. A gene at 59 minutes on the *Escherichia coli* chromosome encodes a lipoprotein with unusual amino acid repeat sequences. J. Bacteriol. **176:**1630–1638.
23. **Iriarte, M., and G. R. Cornelis.** 1995. MyfF, an element of the network regulating the synthesis of fibrillae in *Yersinia enterocolitica*. J. Bacteriol. **177:**738–744.
24. **Iriarte, M., J.-C. Vanooteghem, I. Delor, R. Diaz, S. Knutton, and G. R. Cornelis.** 1993. The Myf fibrillae of *Yersinia enterocolitica*. Mol. Microbiol. **9:**507–520.
25. **Ivanova, A., C. Miller, G. Glinsky, and A. Eisenstark.** 1994. Role of *rpoS* (*katF*) in *oxyR*-independent regulation of hydroperoxidase I in *Escherichia coli*. Mol. Microbiol. **12:**571–578.
26. **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.
27. **Kaasen, I., P. Falkenberg, O. B. Styrvold, and A. R. Strom.** 1992. Molecular cloning and physical mapping of the *otsBA* genes, which encode the osmoregulatory trehalose pathway of *Escherichia coli*: evidence that transcription is activated by KatF (AppR). J. Bacteriol. **174:**889–898.
28. **Kaniga, K., I. Delor, and G. R. Cornelis.** 1991. A wide-host-range suicide vector for improving reverse genetics in Gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. Gene **109:**137–141.
29. **Kolter, R., D. A. Siegele, and A. Tormo.** 1993. The stationary phase of the bacterial life cycle. Annu. Rev. Microbiol. **47:**855–874.
30. **Kowarz, L., C. Coynault, V. Robbe-Saule, and F. Norel.** 1994. The *Salmonella typhimurium katF* (*rpoS*) gene: cloning, nucleotide sequence, and regulation of *spvR* and *spvABCD* virulence plasmid genes. J. Bacteriol. **176:**6852–6860.
31. **Lambert de Rouvroit, C., C. Sluiters, and G. R. Cornelis.** 1992. Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of *Yersinia enterocolitica*. Mol. Microbiol. **6:**395–409.
32. **Lange, R., M. Barth, and R. Hengge-Aronis.** 1993. Complex transcriptional control of the σ^s-dependent stationary-phase-induced and osmotically regulated *osmY* (*csi-5*) gene suggests novel roles for Lrp, cyclic AMP (cAMP) receptor protein-cAMP complex, and integration host factor in the stationary-phase response of *Escherichia coli*. 1993. J. Bacteriol. **175:**7910–7917.
33. **Lange, R., and R. Hengge-Aronis.** 1991. Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. Mol. Microbiol. **5:**49–59.
34. **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 σ^S. J. Bacteriol. **173:**4474–4481.
35. **Lange, R., and R. Hengge-Aronis.** 1994. The *nlpD* gene is located in an operon with *rpoS* on the *Escherichia coli* chromosome and encodes a novel lipoprotein with a potential function in cell wall formation. Mol. Microbiol. **13:**733–743.
36. **Lange, R., and R. Hengge-Aronis.** 1994. The cellular concentration of the σ^s subunit of RNA polymerase in *Escherichia coli* is controlled at the levels of transcription, translation and protein stability. Genes Dev. **8:**1600–1612.

37. **Loewen, P. C., and B. L. Triggs.** 1984. Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. J. Bacteriol. **160:**668–675.
38. **Loewen, P. C., I. von Ossowski, J. Switala, and M. R. Mulvey.** 1993. KatF (σ^S) synthesis in *Escherichia coli* is subject to posttranscriptional regulation. J. Bacteriol. **175:**2150–2153.
39. **McCann, M. P., C. D. Fraley, and A. Matin.** 1993. The putative σ factor KatF is regulated posttranscriptionally during carbon starvation. J. Bacteriol. **175:** 2143–2149.
40. **McCann, M. P., J. P. Kidwell, and A. Matin.** 1991. The putative σ factor KatF has a central role in development of starvation-mediated general resistance in *Escherichia coli*. J. Bacteriol. **173:**4188–4194.
41. **Ménard, R., P. J. Sansonetti, and C. Parsot.** 1993. Nonpolar mutagenesis of the *ipa* genes defines IpaB, IpaC, and IpaD as effectors of *Shigella flexneri* entry into epithelial cells. J. Bacteriol. **175:**5899–5906.
42. **Mikulskis, A. V., I. Delor, V. Ha Thi, and G. R. Cornelis.** 1994. Regulation of the *Yersinia enterocolitica* enterotoxin Yst gene. Influence of growth phase, temperature, osmolarity, pH and bacterial host factors. Mol. Microbiol. **14:**905–915.
43. **Mulvey, M. R., and P. C. Loewen.** 1989. Nucleotide sequence of *katF* of *Escherichia coli* suggests KatF protein is a novel σ transcription factor. Nucleic Acids Res. **17:**9979–9991.
44. **Mulvey, M. R., J. Switala, A. Borys, and P. C. Loewen.** 1990. Regulation of transcription of *katE* and *katF* in *Escherichia coli*. J. Bacteriol. **172:**6713–6720.
45. **Olsen, A., A. Arnqvist, M. Hammar, S. Sukupolvi, and S. Normark.** 1993. The RpoS sigma factor relieves H-NS-mediated transcriptional repression of *csgA*, the subunit gene of fibronectin-binding curli in *Escherichia coli*. Mol. Microbiol. **7:**523–536.
46. **O'Neal, C. R., W. M. Gabriel, A. K. Turk, S. J. Libby, F. C. Fang, and M. P. Spector.** 1994. RpoS is necessary for both the positive and negative regulation of starvation survival genes during phosphatase, carbon, and nitrogen starvation in *Salmonella typhimurium*. J. Bacteriol. **176:**4610–4616.
47. **Pai, C. H., and V. Mors.** 1978. Production of enterotoxin by *Yersinia enterocolitica*. Infect. Immun. **19:**908–911.
48. **Pearson, W. R., and D. J. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
49. **Pepe, J. C., J. L. Badger, and V. L. Miller.** 1994. Growth phase and low pH affect the thermal regulation of the *Yersinia enterocolitica inv* gene. Mol. Microbiol. **11:**123–135.
50. **Sak, B. D., A. Eisenstark, and D. Tonati.** 1989. Exonuclease III and the catalase hydroperoxidase II in *Escherichia coli* are both regulated by the *katF* gene product. Proc. Natl. Acad. Sci. USA **86:**3271–3275.
51. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
52. **Schlensog, V., and A. Böck.** 1991. The *Escherichia coli fdv* gene probably encodes MutS and is located at minute 58.8 adjacent to the *hyc-hyp* gene cluster. J. Bacteriol. **173:**7414–7415.
53. **Shaw, W. V.** 1975. Chloramphenicol acetyltransferase from chloramphenicol-resistant bacteria. Methods Enzymol. **43:**737–755.
54. **Siegele, D. A., and R. Kolter.** 1992. Life after log. J. Bacteriol. **174:**345–348.
55. **Small, P., D. Blankenhorn, 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.
56. **Sory, M. P., P. Hermand, J. P. Vaerman, and G. R. Cornelis.** 1990. Oral immunization of mice with a live recombinant *Yersinia enterocolitica* O:9 strain that produces the cholera toxin B subunit. Infect. Immun. **58:**2420–2428.
57. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82:**1074–1078.
58. **Tanaka, K., Y. Takayanagi, N. Fujita, A. Ishihama, and H. Takahashi.** 1993. Heterogeneity of the principal σ factor in *Escherichia coli*: the *rpoS* gene product, σ^{38}, is a second principal σ factor of RNA polymerase in stationary-phase *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**3511–3515.
59. **Touati, E., E. Dassa, and P. L. Boquet.** 1986. Pleiotropic mutations in *appR* reduce pH2.5 acid phosphatase expression and restore succinate utilisation in CRP-deficient strains of *Escherichia coli*. Mol. Gen. Genet. **202:**257–264.
60. **Vicente, M., S. R. Kushner, T. Garrido, and M. Aldea.** 1991. The role of the 'gearbox' in the transcription of essential genes. Mol. Microbiol. **5:**2085–2091.
61. **Wang, A.-Y., and J. E. Cronan, Jr.** 1994. The growth phase-dependent synthesis of cyclopropane fatty acids in *Escherichia coli* is the result of an RpoS(KatF)-dependent promoter plus enzyme instability. Mol. Microbiol. **11:**1009–1017.
62. **Weichart, D., R. Lange, N. Henneberg, and R. Hengge-Aronis.** 1993. Identification and characterization of stationary phase-inducible genes in *Escherichia coli*. Mol. Microbiol. **10:**407–420.
63. **Yim, H. H., R. L. Brems, and M. Villarejo.** 1994. Molecular characterization of the promoter of *osmY*, an *rpoS*-dependent gene. J. Bacteriol. **176:**100–107.