Cloning, Nucleotide Sequence, and Regulation of *katE* Encoding a σB-Dependent Catalase in *Bacillus subtilis*

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A σB-dependent stress gene of *Bacillus subtilis* was localized downstream of the *licS* gene. The predicted amino acid sequence exhibited a significant similarity to the sequence of the *katE*-encoded catalase HPII of *Escherichia coli*, and we designated it the open reading frame *katE*. In a *B. subtilis katE* mutant, catalase 2 could not be detected. The amount of *katE*-specific mRNA was increased after heat, salt, or ethanol stress or after glucose starvation in a σB-dependent manner. As in *E. coli*, the transcription of the *katE* gene in *B. subtilis* was unaffected by the addition of H_2O_2 to exponentially growing cells. In contrast, the *katA* gene encoding catalase 1 of *B. subtilis* showed an induction pattern different from that of *katE*; *katA* expression was strongly increased by oxidative stress. The similarity between *E. coli* σS-dependent genes and *B. subtilis* σB-dependent genes suggests that both may confer multiple stress resistance to stationary-phase cells.

In its natural environment *Bacillus subtilis* is often exposed to limiting amounts of nutrients and high salt concentrations as well as alterations in pH and temperature. As a consequence, *B. subtilis* is well equipped with a stress defense machinery to cope with these growth-restricting conditions. The very strong induction of a typical set of proteins in *B. subtilis*, which we call general stress proteins, occurs within the first minutes after the exposure of cells to stress (21, 22, 44, 53) and is therefore one of the earliest responses to environmental changes.

The alternative sigma factor σB, which is one of 10 sigma factors in *B. subtilis* identified so far (33), appears to be an important regulator of the synthesis of stress-induced proteins (10, 52, 53). The increased activity of σB after stress or early in stationary phase has been correlated with the increased synthesis of σB-dependent stress proteins (5, 9–11, 53). In a *sigB* deletion mutant these proteins fail to be induced. Nevertheless, the mutation of *sigB* did not result in an increased sensitivity to stress or starvation. Therefore, the physiological role of σB under these conditions remains obscure (17).

In order to understand the role of σB, it is necessary to investigate the function and regulation of genes controlled by *sigB* in more detail. *ctc* (20), *csbA* (12), and *sigB* (9, 10, 25) were the first genes shown to be subject to σB-dependent regulation. Both *ctc* and *sigB* are strongly induced upon entry into stationary phase and after heat, salt, or ethanol stress (4, 9, 53). Recently, we have shown that the *gsiB* gene, which was originally identified as a gene induced by glucose starvation (37), belongs to the general stress genes in *B. subtilis* and requires σB for its induction in response to various stimuli (32, 53). Unfortunately, the physiological functions of the gene products of *csbA*, *ctc*, and *gsiB* have not yet been identified.

Recently, new *csb* loci have been identified by a genetic approach (10). The expression of these *csb* loci is either completely or partially dependent on σB. One of these loci is *gtaB*

(52), which encodes UDP-glucose pyrophosphorylase. The enzyme catalyzes the synthesis of UDP-glucose.

In this communication we describe the identification of an additional *sigB*-dependent gene which is homologous to *katE* of *Escherichia coli* (54). The *katE* gene encodes catalase HPII in *E. coli* and is induced when cells enter stationary phase. The expression of *katE* is regulated by the alternative sigma factor σS and is unaffected by H_2O_2 (38, 47, 54). Both σS and HPII are involved in the resistance of stationary-phase cells against oxidative stress (14, 23, 30).

We investigated the regulation of the σB-dependent *katE* homologous gene of *B. subtilis* under different stress conditions. A comparison of the induction patterns of *katE* and *katA*, which encodes catalase 1 in *B. subtilis* (7, 29), shows that the two catalase genes are subject to different regulatory mechanisms.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The bacterial strains and plasmids used in this study are listed in Table 1. *E. coli* DH5α and RR1 were routinely grown in a complex medium and used as hosts for DNA manipulation. *B. subtilis* strains were cultivated under vigorous agitation at 37°C in a synthetic medium described previously (50). Exponentially growing cells of *B. subtilis* were shifted from 37 to 48°C, achieving a heat shock, or exposed to either 4% (wt/vol) NaCl, 4% (vol/vol) ethanol, or 0.001 or 0.0002% (vol/vol) H_2O_2. Starvation for glucose was achieved by cultivating bacteria in the synthetic medium with growth-limiting amounts of glucose (0.05%, wt/vol).

Construction of a *katE* insertional mutant. The 865-bp *Mlu*I-*Hin*dIII fragment of pRB31 containing an internal part of *katE* of *B. subtilis* was filled in with the Klenow fragment of DNA polymerase I and ligated with the *Eco*RV-digested integration vector pJH101, generating the plasmid pJHKE2. This plasmid was used to transform competent cells of *B. subtilis* IS58. Chloramphenicol-resistant colonies were selected on agar plates with 5 μg of chloramphenicol per ml. The integration of the plasmid into the chromosome was verified by Southern blot analysis. The resulting mutant, MkatE2, was used to clone the C-terminal part of *katE*. For this purpose, chromosomal DNA of *B. subtilis* MkatE2 was digested with *Aat*II, religated, and transformed into competent cells of *E. coli* DH5α, yielding plasmid pJHKMA2 (Fig. 1).

Sequence determination of *katE*. For sequencing, a 1,705-bp *Bgl*II-*Hin*dIII fragment and a 2,900-bp *Sal*I-*Cla*I fragment from pRB31 were subcloned into the vector pBluescriptIIKS⁺, generating the plasmids pKSKBH2 and pKSKSC2, respectively. DNA sequencing was carried out by the dideoxy chain-termination method of Sanger et al. (46). Both strands were sequenced with plasmid DNA as a template. The sequence was determined by primer walking with the plasmids

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or description	Reference or source
Strains		
E. coli		
DH5α	F⁻ Φ80d*lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *deoR recA1 endA1 hsdR17* (r$_K^-$ m$_K^+$) *supE44 thi-1 gyrA69*	45
RR1	F⁻ *mcrB mrr hsdS20* (r$_B^-$ m$_B^-$) *ara14 proA2 lacY1 leu galK2 rpsL20* (Sm^r) *xyl5 mtl1 supE44*	45
B. subtilis		
IS58	*trpC2 lys-3*	49
BGH1	*trpC2 lys-3 sigB*::Δ*Hin*dIII-*Eco*RV::*cat*	32
MkatE2	*trpC2 lys-3 katE*::pJHKE2	This study
Plasmids		
pBluescriptIIKS⁺	Cloning vector, Ap	Stratagene
pJH101	Integrative plasmid, Ap^r Tc^r Cm^r	19
pRB31	pBR322 containing a 5.6-kb *Hin*dIII fragment of *B. subtilis* DNA	56
pKSKBH2	pBluescriptIIKS⁺ containing the 1,705-bp *Bgl*II-*Hin*dIII fragment from pRB31	This study
pKSKSC2	pBluescriptIIKS⁺ containing the 2.9-kb *Sal*I-*Cla*I fragment of pRB31	This study
pKSKBE13	pBluescriptIIKS⁺ containing the 1,220-bp *Bgl*II-*Eco*RI fragment of pRB31	This study
pJHKE2	pJH101 containing the 865-bp *Mlu*I-*Hin*dIII fragment of pRB31	This study
pJHKMA2	Obtained after plasmid rescue from *Aat*II-digested DNA of *B. subtilis* MkatE2	This study
pKSK19/5	pBluescriptIIKS⁺ containing the *Eco*RI-*Sal*I-digested PCR product of *katA*	This study

pKSKBH2, pKSKSC2, and pJHKMA2 or with a set of subclones of pKSKBH2 generated by digestion with restriction endonucleases.

Slot blot analysis. Total RNA of *B. subtilis* IS58 was isolated from exponentially growing or stressed cells by the acid phenol method described previously (31, 53). Serial dilutions of total RNA were transferred onto a positively charged nylon membrane by slot blotting and hybridized with digoxigenin-labeled RNA probes according to the instructions of the manufacturer (Boehringer Mannheim). The chemiluminograms were quantified with a personal densitometer from Molecular Dynamics. Induction rates were calculated by setting the value of the control to 1.

The hybridization analysis specific for *katE* mRNA was performed with a digoxigenin-labeled RNA probe which was synthesized in vitro with T3 polymerase from linearized plasmid pKSKBE13. This plasmid is derived from pBluescriptIIKS⁺ and contains a 1,220-bp *Bgl*II-*Eco*RI fragment of pRB31 coding for the N-terminal and control regions of *katE*.

For the analysis of the transcription, the *katA* gene was amplified from chromosomal DNA with oligonucleotide primers P1 (5′ TGAACTTGGCTCTGCTG ACAC) and P2 (5′ GCCGTTATCATCAAAACGC) derived from the nucleotide sequence of *katA* (7). The PCR product was cut with *Eco*RI and *Sal*I and ligated with pBluescriptIIKS⁺ digested with the same enzymes, resulting in plasmid pKSK19/5. The hybridization analysis specific for *katA* mRNA was carried out with a digoxigenin-labeled RNA probe synthesized in vitro with T3 polymerase from the *Eco*RI-linearized plasmid pKSK19/5.

Primer extension and Northern (RNA) blot analysis. The 5′ end of the *katE* mRNA was identified by the primer extension method as described previously (55). The oligonucleotides 5′ TTTGTCGTCATTTTCTTCCCGC and 5′ TCAT GCTTGCCATACTCGG labeled with [γ-³²P]ATP at the 5′ end were used as primers.

Northern blot analysis was performed as described previously (55) and with the same probe used for slot blot analyses.

Visualization of catalase activities on polyacrylamide gels. Cells were harvested and sonically disrupted. Protein extracts were separated on 10% polyacrylamide gels run according to standard protocols (3), except that for the separating gel Tris-HCl (pH 8.1) was used. Catalase activity was visualized on the gel by the method described previously (13, 28).

General methods. Plasmid isolation, restriction enzyme analysis, transformation of *E. coli*, ligation of DNA fragments, and filling in the recessed 3′ termini by the Klenow fragment of DNA polymerase I were performed according to standard protocols (45). Chromosomal DNA from *B. subtilis* was isolated as described by Msadek et al. (36). The transformation of *B. subtilis* was carried out with natural competent cells (24).
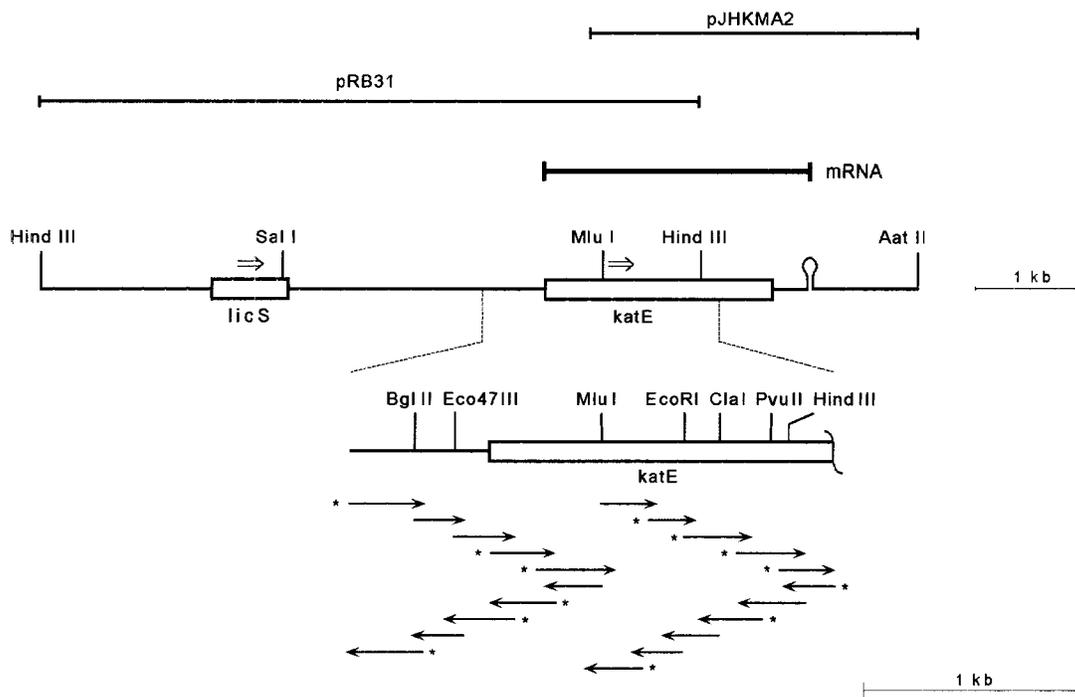
FIG. 1. Physical map of the *katE* region of the chromosome. The arrows at the bottom describe the strategy used to sequence the *katE* gene and indicate the direction and extent of elongation in the sequencing reactions. The restriction map of the *Bgl*II-*Hin*dIII fragment of pRB31 was used to generate subclones of portions of the fragment which were used for sequencing. An asterisk at the end of some arrows indicates that the sequence was initiated with synthetic primers with sequences that correspond to the *katE* sequence. A putative terminator is indicated by a loop downstream of *katE*.

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                                                                                                      *
   1  TGGTAATCATATAGGTTGTTGTCCCATCTCCGTCCAGCGAAATGGTCATGGTCAGAACCGCTGTGCCTACGGCGATTTTTAACGGATCTCCTTTGACAAACGATAAGATTTTGGCAATGA  120
                                                                                                    S2
                                                                                                     .BglII
 121  GAGGATCAAACAGGCCTGAGTCAATCATAATGCCGAAATACAGGATGGCGAATAACAGCATGATACCGGTAGGGGCGAGGTCTGTAACGCCCTGAATCATCATCTCGCCGAGATCTTTGC  240

 241  CAAATCCGCTGATCAGGGCAAACACAATAGGAACGACAATCAAAGCAATAAGAGCGGAAAGCCGGTTAGACATAATAAGGTACATAAAGACAATCATCATCACAAAACCGAGTATGGCAA  360
                                         Eco47III
 361  GCATGATATGACCTCCCCTATTCAACAGCTTTTTTGGTAAGCGCTTCCATCCTTTCTTTCTGTGAAGAAGGCTTATTTCAAATGTGTATGACCGCATATTTATATGTAGACCAAAAATCC  480
                                                                     *
 481  TAATGAGCGGATTAGCAGTTTATATGAAGAACGCCACGGGTAAATGTGCTGTAGAATGTTTCGTTTTAAAAAGGGGGAGCAGACATGAGTGATGACCAAAACAAACGTGTAAATGAACAC  600
                    -35             -10          S1                    SD          M  S  D  D  Q  N  K  R  V  N  E  H
                                                                                    katE --->
 601  TCAAAGGACGAGCAGCTTGAGCAGTACAGGACCGATAACAGCGGGAAGAAAATGACGACAAACCAAGGCTTGCGAGTGTCAGAGGATGAGCACTCGTTAAAAGCCGGGGTTCGCGGCCCG  720
      S  K  D  E  Q  L  E  Q  Y  R  T  D  N  S  G  K  K  M  T  T  N  Q  G  L  R  V  S  E  D  E  H  S  L  K  A  G  V  R  G  P
 721  ACGCTGATGGAGGATTTTCATTTCCGGGAGAAAATGACGCACTTTGACCATGAGAGGATTCCGGAGCGCGTTGTGCATCGCGCGGATTTGGCGTGCATGGCTTTTTTCAGGTTTACGAA  840
      T  L  M  E  D  F  H  F  R  E  K  M  T  H  F  D  H  E  R  I  P  E  R  V  V  H  A  R  G  F  G  V  H  G  F  F  Q  V  Y  E
 841  CCAATGACGGAATACACACGGGCCAAATTTCTTCAGGACCCTTCGGTGAAGACACCGGTATTTGTCCGTTTTTCTACGGTTgcgGGTTCTAAAGGCTCCGCAGACACGGTGCGGGATGCC  960
      P  M  T  E  Y  T  R  A  K  F  L  Q  D  P  S  V  K  T  P  V  F  V  R  F  S  T  V  A  G  S  K  G  S  A  D  T  V  R  D  A
                                                                                                              .MluI
 961  AGAGGCTTTGCGACAAAATTTTATACTGAAGAAGGAAACTATGACCTTGTGGGAAATAACATTCCGGTGTTTTTCATTCAGGACGCAATCAAATTTCCGGATTTGGTTCACGCGTTTAAG  1080
      R  G  F  A  T  K  F  Y  T  E  E  G  N  Y  D  L  V  G  N  N  I  P  V  F  F  I  Q  D  A  I  K  F  P  D  L  V  H  A  F  K
1081  CCTGAGCCACACAATGAAATGCCTCAGGCTGCGACTGCTCACGATACATTTTGGGATTTTGTCGCCAATAACCCTGAGTCTGCCCACATGGTGATGTGGACGATGTCTGACAGAGGCATC  1200
      P  E  P  H  N  E  M  P  Q  A  A  T  A  H  D  T  F  W  D  F  V  A  N  N  P  E  S  A  H  M  V  M  W  T  M  S  D  R  G  I
1201  CCGCGCAGCTACAGAATGATGGAAGGCTTCGGCGTTCACACGTTCCGTTTTGTCAATGAACAAGGAAAGGCACGTTTCGTGAAATTTCACTGGAAGCCGGTGCTCGGCGTGCATTCTCTC  1320
      P  R  S  Y  R  M  M  E  G  F  G  V  H  T  F  R  F  V  N  E  Q  G  K  A  R  F  V  K  F  H  W  K  P  V  L  G  V  H  S  L
1321  GTATGGGATGAAGCGCAAAAAATTGCCGGGAAAGATCCAGATTTCCACCGCCGTGACCTGTGGGAAACAATTGAAAACGGCGGCAAGGTTGAATACGAGCTCGGCGTGCAGATGATTGAT  1440
      V  W  D  E  A  Q  K  I  A  G  K  D  P  D  F  H  R  R  D  L  W  E  T  I  E  N  G  G  K  V  E  Y  E  L  G  V  S  Q  M  I  D
                        EcoRI
1441  GAGGAAGATGAATTCAAGTTTGATTTTGATATTCTTGACCCAACTAAGCTGTGGCCGGAAGAGCTTGTGCCGGTGAAGATCATTGGAAAAATGACGCTGAACCGCAATCAGGATAATGTG  1560
      E  E  D  E  F  K  F  D  F  D  I  L  D  P  T  K  L  W  P  E  E  L  V  P  V  K  I  I  G  K  M  T  L  N  R  N  Q  D  N  V
                                                                   ClaI
1561  TTTGCGGAAACAGAACAAGTCGCATTCCACCCGGGAAATGTCGTGCCGGGCATCGATTTTACCAATGACCCGCTATTGCAGGGGCGGCTTTTCTCTTATACGGATACACAGCTCATCCGT  1680
      F  A  E  T  E  Q  V  A  F  H  P  G  N  V  V  P  G  I  D  F  T  N  D  P  L  L  Q  G  R  L  F  S  Y  T  D  T  Q  L  I  R
1681  CTTGGCGGCCCGAACTTCCATGAGATTCCGATTAACCGGCCGGTCTGTCCATTCCATAACAACCAATACGACGGCTATCACCGGATGACGATCAATAAGGGACCTGTCGCTTATCATAAA  1800
      L  G  G  P  N  F  H  E  I  P  I  N  R  P  V  C  P  F  H  N  N  Q  Y  D  G  Y  H  R  M  T  I  N  K  G  P  V  A  Y  H  K
                              PvuII
1801  AACTCACTGCAAAACAATGATCCGTCGCCTGCGACAGCTGAAGAAGGCGGCTACGTCCACTATCAGGAAAAAGTCGAGGGCAAAAAAATCCGCCAGCGAAGCGACAGCTTCAACGATTAT  1920
      N  S  L  Q  N  N  D  P  S  P  A  T  A  E  E  G  G  Y  V  H  Y  Q  E  K  V  E  G  K  K  I  R  Q  R  S  D  S  F  N  D  Y
                      HindIII
1921  TACTCACAGGCGAAGCTTTTCTGGAACAGCATGTCTCCTGTGGAGAAACAGCACATCATTTCTGCTTTCTGCTTCGAGGTCGGGAAGGTCAAAAGCAAAGACGTACAGCGGCAGGTCGTT  2040
      Y  S  Q  A  K  L  F  W  N  S  M  S  P  V  E  K  Q  H  I  I  S  A  F  C  F  E  V  G  K  V  K  S  K  D  V  Q  R  Q  V  V
2041  GATGTCTTCAGCAATGTCGATGCAGATTTAGCAGAAGAAATCGCCAAAGGAGTAGGCGTGGCGGCGCCCGCGAAGCGTAAAGCATCTAAAGAAATTCTTACTTCGCCTGCGTTAAGCCAA  2160
      D  V  F  S  N  V  D  A  D  L  A  E  E  I  A  K  G  V  G  V  A  A  P  A  K  R  K  A  S  K  E  N  L  T  S  P  A  L  S  Q
2161  GCCCGTACAGTGA  2172
      A  R  T  V
```
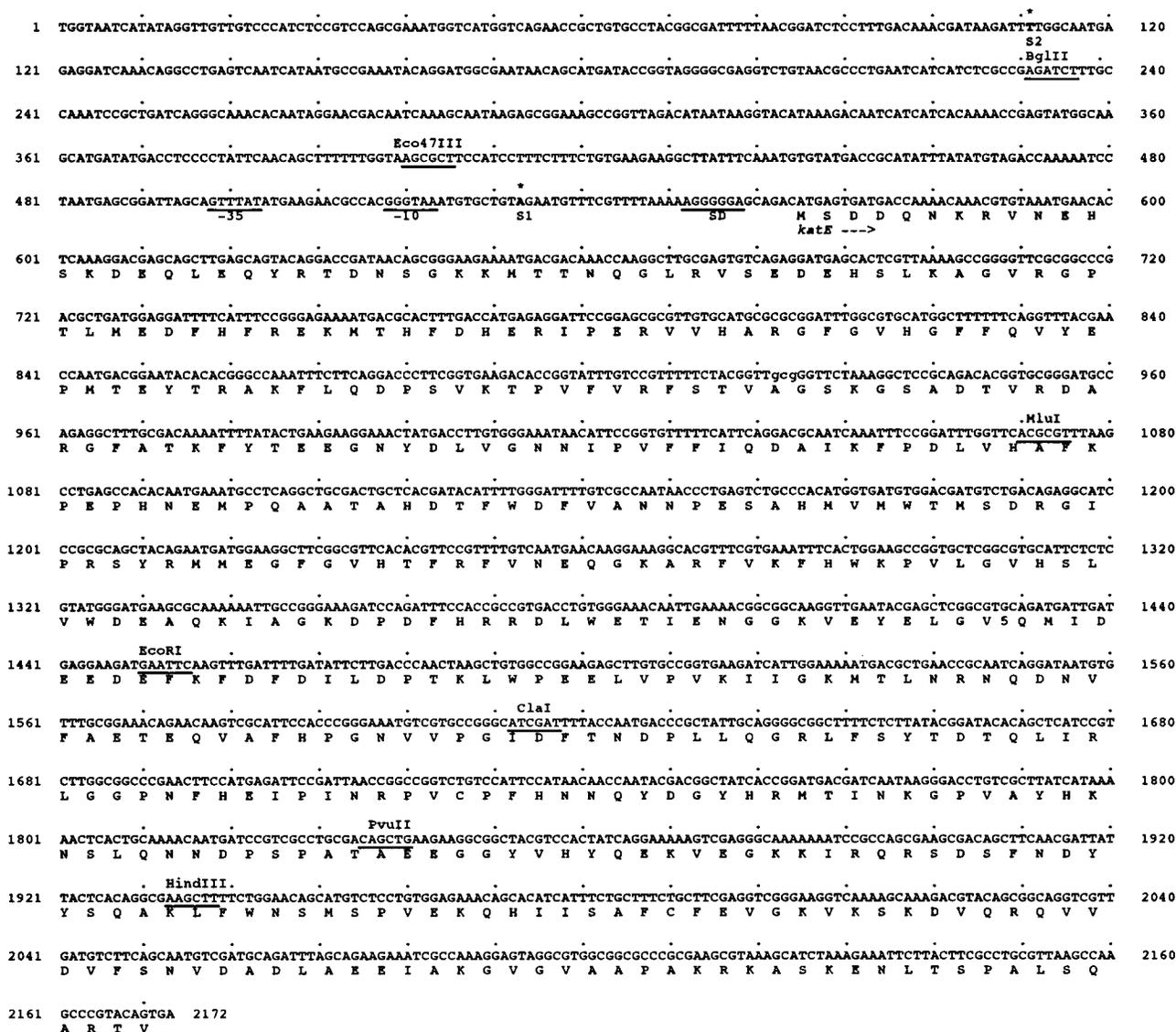
FIG. 2. Nucleotide sequence of the regulatory region and the N terminus of the *katE* locus of *B. subtilis*. The deduced amino acid sequence of the *katE* gene product is given underneath the DNA sequence. The putative Shine-Dalgarno (SD) sequence, the potential −35 and −10 regions of the *sigB*-dependent promoter, and the restriction enzyme sites used in this study are underlined. The two possible transcription start sites S1 and S2 are indicated by asterisks and printed in boldface type.

Computer analysis of sequence data. The sequence data manipulations were performed with the Genetic Computer Group sequence analysis software package.

Nucleotide sequence accession number. The nucleotide sequence databases reported in this paper appear in the EMBL and GenBank nucleotide sequence databases under the accession number X85182.

RESULTS

Cloning and nucleotide sequence of *katE* in *B. subtilis*. Sequence analysis downstream of *licS* (39), encoding β-1.3-1.4-endoglucanase, revealed an open reading frame of at least 1,606 nucleotides (Fig. 1 and 2). A putative ribosome binding site (5′ AGGGGGA) was found 7 bp upstream of the initiation codon (Fig. 2). While the sequencing was in progress, Fujita and coworkers cloned and sequenced this region as a part of the *B. subtilis* genome sequencing project. We have learned that the full open reading frame contains 2.06 kb and codes for a potential protein of 77 kDa. A putative terminator structure was identified 300 bp downstream of the stop codon of the open reading frame (19a).

A comparison of the deduced amino acid sequence of the putative gene product with sequences of known proteins revealed significant similarities to large regions of numerous prokaryotic and eukaryotic catalases. These included catalases from *E. coli* (54), *Bacillus firmus* (42), *Bacillus subtilis* (7), *Oryza sativa* (35), humans (41), and mouse (48). Loewen (27) mapped a locus which affects catalase 2 levels at this position and designated the locus *katB*. By analogy with the *E. coli* gene, however, we have named the *B. subtilis* coding sequence *katE*. Interestingly, the degree of similarity among these enzymes is stronger within the N terminus of the amino acid sequences than within the C terminus. The potential heme-binding site is

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                           *
Bs2   DFTNDPLLQG  RLFSYTDTQL  IRLGGPNFHE

Bf    DFTNDPLLQG  RLFSYTDTQL  IRLGGPNFHE

Ec    DFTNDPLLQG  RLFSYTDTQI  SRLGGPNFHE

Bs1   DVSPDKMLQG  RLFAYHDAHR  YRV.GANHQA

Os    YYSDDKMLQC  RVFAYADTQR  YRL.GPNYLM

Mm    EPSPDKMLQG  RLFAYPDTHR  HRL.GPNYLQ

Hs    EASPDKMLQG  RLFAYPDTHR  HRL.GPNYLH
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FIG. 3. Alignment of the heme-binding region of the *katE* gene product of *B. subtilis* (Bs2) with sequences of other catalases, namely HPII of *E. coli* (Ec) (54), the *katA* gene product of *B. firmus* (Bf) (42), catalase 1 of *B. subtilis* (Bs1) (7), catalase of *Oryza sativa* (Os) (35), catalase of mouse (Mm) (48), and human catalase (Hs) (41). The putative heme-binding region is printed in boldface type. The asterisk indicates the heme-binding site.

strongly conserved between these proteins and was found at position 365 of KatE of *B. subtilis* (Fig. 3).

Effects of disruption of *katE* on catalase activity. To confirm that *katE* encodes a catalase, protein extracts of exponentially growing cells of the wild-type strain IS58 and the *katE* mutant MkatE2 were separated electrophoretically on native polyacrylamide gels. Catalase activity bands were visualized as described in Materials and Methods. In crude extracts of the wild-type strain, two catalase species were found (Fig. 4), which is in accordance with results by Loewen and Switala (28). Catalase 1, which is encoded by *katA* (7, 29), shows a higher activity during exponential growth phase than does catalase 2. In the *katE* mutant, only catalase 1 was present. Therefore, we conclude that *katE* either codes for *B. subtilis* catalase 2 or is required for catalase 2 synthesis.

Transcription of *katE* and *katA* in response to different stresses. The expression of *katE* in *E. coli* is controlled by σ^S and increases as cells enter stationary phase (38, 47). Therefore, we investigated the influence of various stress conditions on the expression of *katE* in *B. subtilis*. Slot blot filters with total RNA isolated from exponentially growing and glucose-starved cells of *B. subtilis* were prepared and hybridized with a digoxigenin-labeled RNA probe specific for *katE* as described in Materials and Methods. The hybridization signals showed a 50- to 70-fold increase in *katE*-specific mRNA levels when cells entered the stationary phase. A similar induction of *katE* was observed after exposing cells to heat stress (80-fold increase), salt stress (50-fold increase), or ethanol stress (40- to 50-fold increase) (Fig. 5). However, the gene was not induced by 0.001 or 0.0002% H_2O_2 (Fig. 5).

Northern blot experiments performed using the same *katE*-specific RNA probe revealed a transcript of 2.4 kb (Fig. 6). If transcription of *katE* initiates just upstream of the coding sequence, it would appear to terminate 300 bp downstream of the stop codon presumed by Fujita and coworkers on the basis of the sequencing results (19a). The relative amount of the transcript was strongly increased after heat stress (Fig. 6).

We also investigated the influence of different stress conditions and glucose starvation on the expression of *katA*, which is controlled by the vegetative sigma factor σ^A (8). Although, there was no increase of *katA*-specific mRNA after heat, salt, and ethanol stress, we could measure about a 140-fold increase after the addition of H_2O_2 to growing cells and a weak increase (of about 10-fold) when cells entered the stationary phase because of glucose exhaustion (Fig. 5).

According to its induction pattern, *katE* belongs to the group of general stress genes in *B. subtilis* (21, 53). Recently, it has been established that the alternative sigma factor σ^B is required for the induction of a large number of general stress genes (10, 53). On this account, we measured the expression of *katE* in the *sigB* deletion mutant BGH1 in slot blot experiments and found no induction of *katE* after heat, ethanol, or salt stress or after glucose starvation (data not shown).

Determination of the transcriptional start site of *katE*. The 5′ end of *katE* mRNA (S1) was detected 30 bp upstream of the start codon of *katE* by the primer extension technique. In the *B. subtilis* wild-type strain IS58, transcription apparently initiating at this site was induced by heat or ethanol stress (Fig. 7) or by salt stress or glucose starvation (data not shown). However, no signal was detected when RNA isolated from stressed cells of the *sigB* deletion mutant BGH1 was used (results are shown for heat and ethanol stress in Fig. 7 but data are not shown for salt stress and glucose starvation). The potential -10 and -35 regions of a RNA polymerase binding site upstream of the start site are similar to those of known σ^B-dependent genes (Fig. 7; Table 2).

A second potential start site of transcription (S2) was found 454 bp upstream of the start codon. Neither the imposition of stress nor the deletion of *sigB* changed the strength of this hybridization signal (Fig. 7). The sequence upstream of this potential start site did not reveal any substantial similarities with consensus sequences of the known sigma factors of *B. subtilis*.

DISCUSSION

In this work we present the identification of an additional σ^B-dependent stress gene of *B. subtilis*. It is localized downstream of the previously described β-glucanase gene, *licS* (39), at 335° of the *B. subtilis* genetic map (1). Because of the great similarity of the deduced amino acid sequence of the gene to HPII of *E. coli* and other known catalases of prokaryotes and eukaryotes, it was designated *katE*.

In *B. subtilis* the presence of two catalases was postulated on the basis of the observation of two main activity bands on nondenaturing polyacrylamide gels (28). Catalase 1 is found in vegetative cells; catalase 2 is the only catalase found in purified spores (28). Since mutations in *katE* lead to a loss of catalase 2 activity, it is very likely that the *katE* gene codes for
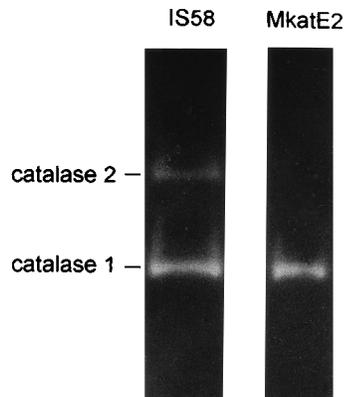

FIG. 4. Catalase activities in crude extracts of the wild-type strain IS58 and *katE* mutant MkatE2 of *B. subtilis*. Samples containing 100 μg of protein were loaded on 10% polyacrylamide gels and separated electrophoretically under nondenaturing conditions. The gel was then stained for catalase by the method of Clare et al. (13).
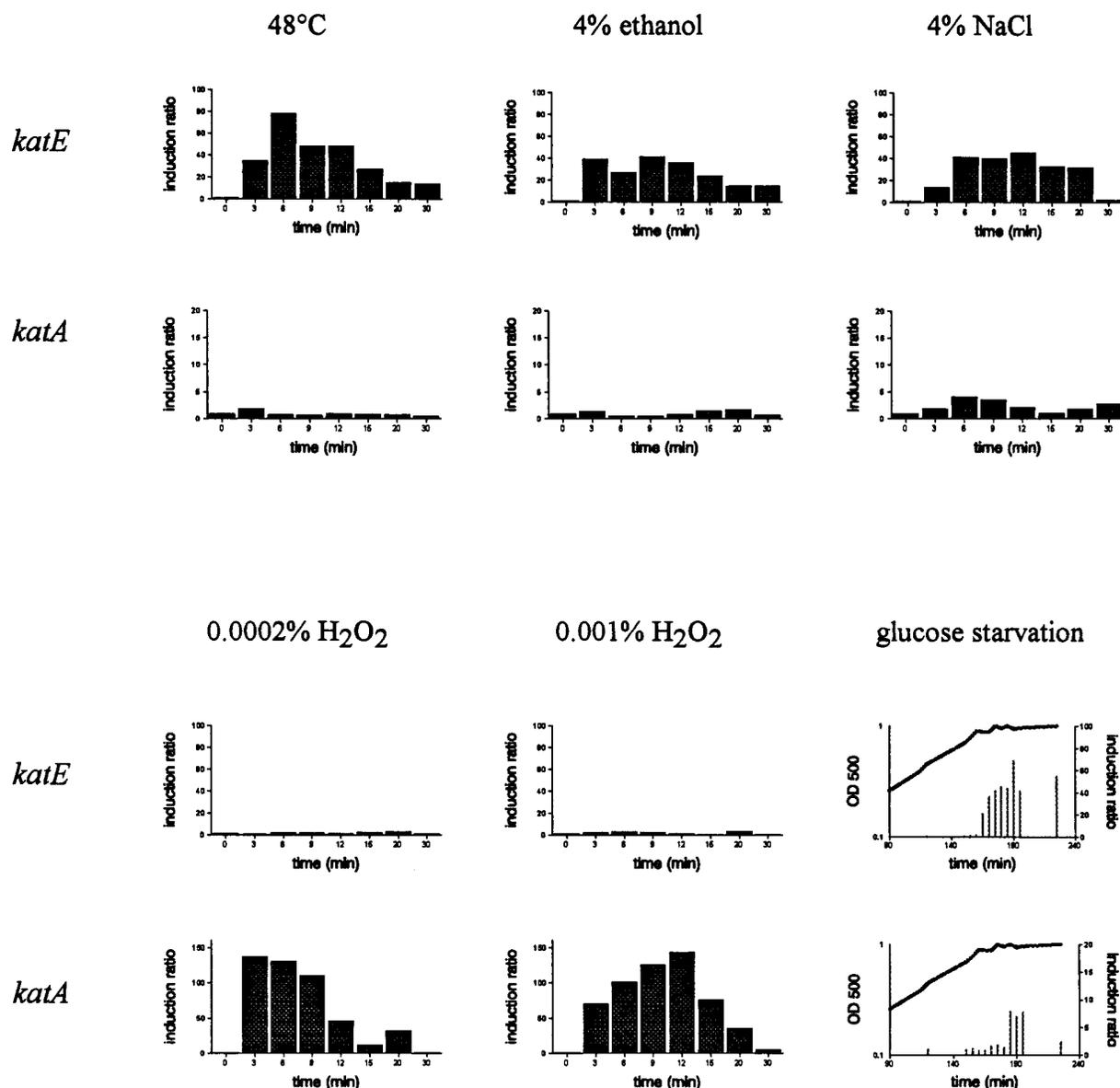
FIG. 5. Schematic representation of changes in the mRNA level of *katE* and *katA* after imposition of various stresses. Total RNA was prepared from *B. subtilis* IS58 before (0 min) and at different times after exposure to stress (3, 6, 9, 12, 15, 20, and 30 min) or, in the case of glucose starvation, from exponentially growing and glucose-starved cells. Serial dilutions of total RNA were blotted onto a positively charged nylon membrane and hybridized with the digoxigenin-labeled antisense RNA probes specific for the corresponding genes. The hybridization signals were quantified with a personal densitometer. Induction ratios were calculated by setting the value of the control (at 0 min) to 1. The induction rates of the mRNA of the two genes are shown (shaded bars). OD 500, optical density at 500 nm.

catalase 2. The *katB* locus identified by Loewen (27) shows the same effect and is probably identical to the *katE* locus. The expression pattern of *katE* in *B. subtilis* is similar to that of *katE* in *E. coli*. We detected an increase in *katE*-specific mRNA when cells enter stationary growth phase, and almost no induction was found after the addition of H_2O_2 to exponentially growing cells. In addition, the gene is induced after ethanol or salt stress and, in contrast to *E. coli*, also after heat stress.

According to its induction pattern, *katE* in *B. subtilis* belongs to the group of general stress genes (22, 53) and its stress induction depends on the alternative sigma factor σ^B. The very strong increase in the amount of *katE* mRNA by heat, ethanol, or salt stress as well as during starvation for glucose is probably due to an increased frequency of transcription
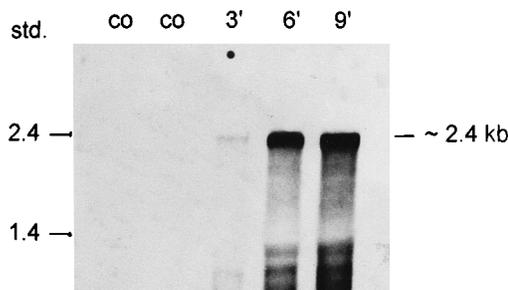
FIG. 6. Northern blot analysis. RNA was isolated from *B. subtilis* IS58 at 37°C (co) and 3, 6, and 9 min (3', 6', and 9', respectively) after a temperature shift to 48°C. The filter was hybridized with a digoxigenin-labeled RNA probe specific for *katE* (see Materials and Methods). std., molecular weight standards (in kilobases).
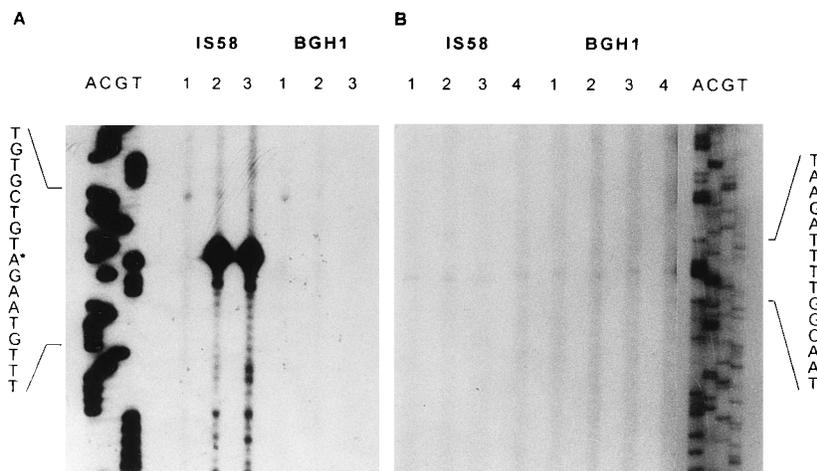
FIG. 7. Mapping of the 5′ end of the *katE* mRNA by primer extension analysis. RNA was isolated from *B. subtilis* IS58 and BGH1 before (lanes 1) and 6 min after heat shock (lanes 2), ethanol stress (lanes 3), and salt stress (lanes 4). Equal amounts of total mRNA (5 μg) were used for the primer extension analysis. The potential start sites of transcription S1 (A) and S2 (B) are marked with asterisks. The oligonucleotide 5′ TTTGTCGTCATTTTCTTCCCGC was used for mapping S1, and 5′ TCATGCTTGCCATACTCGG was used for mapping S2. Lanes A, C, G, and T show the dideoxy sequencing ladder obtained with the appropriate primer.

initiation at a σ^B-dependent promoter (Fig. 7). In a *sigB* deletion mutant we could not detect any hybridization signals at this site (Fig. 7). So far only the σ^B-dependent promoter of the *ctc* gene has been well characterized, and five bases within the proposed −35 and −10 regions (Table 2) have been shown to be very important for promoter activity in vitro and in vivo (43, 51). Interestingly, these bases are conserved in all known σ^B-dependent promoters (Table 2). From these promoter sequences, we derived a consensus sequence of σ^B-dependent promoters. The promoter of *katE* matches the consensus sequence in five of six bases in the −35 region as well as in the −10 region.

The second catalase in *B. subtilis*, catalase 1, is encoded by *katA* (7, 29). The expression of *katA* is increased by oxidative stress and during stationary phase (8). It was presumed that the product of *katA* is responsible for both the inducible resistance of exponentially growing cells and the nonspecific resistance seen in stationary phase (6, 8). We confirmed the strong increase of *katA* expression evoked by an oxidative stress but detected only a weak increase in *katA*-specific mRNA in stationary phase.

Since neither the *katE* gene (Fig. 5) nor the *sigB* gene (9, 18) is induced when exponentially growing cells are treated with sublethal concentrations of H_2O_2, we suspect that neither σ^B nor catalase 2 is involved in the inducible resistance of *B.*

subtilis against H_2O_2. Preliminary experiments have shown that both *sigB* and *katE* mutants are resistant to lethal concentrations of H_2O_2 in the exponential growth phase when the cells were pretreated with sublethal concentrations of H_2O_2 (18). Additional investigations are necessary, however, in order to elucidate the physiological role of different catalases in conferring specific (H_2O_2-induced) and nonspecific (induced in the stationary growth phase) resistance to oxidative stress (6, 15, 16). We have preliminary evidence that both *katE* and *katA* are involved in the stationary phase resistance against oxidative stress in *B. subtilis*.

Furthermore, this study shows that *katE* in *B. subtilis* and *katE* in *E. coli* are regulated in a similar way. Both genes are controlled by alternative sigma factors σ^B and σ^S, respectively, which are known to regulate a number of stationary phase-induced genes. Whereas little is known about the function of σ^B-dependent genes in *B. subtilis*, some of the proteins belonging to the *rpoS* regulon in *E. coli* are well characterized (for review see reference 23). The main physiological role of these σ^S-dependent gene products is to confer multiple stress resistance upon nongrowing *E. coli* cells. It is tempting to speculate that in *B. subtilis* σ^B plays a similar role during stationary phase.

TABLE 2. Promoter regions of known *sigB*-dependent genes[a]

Gene or operon and consensus (reference)	−35 Region sequence	Length of intervening sequence	−10 Region sequence	Length of intervening sequence	Start site and flanking bases
sigB operon (25)	AGGTTTAA	N_{14}	GGGTAT	N_9	TA*G
ctc (34)	AGGTTTAA	N_{14}	GGGTAT	N_{10}	TA*G
csbA (12)	GTGATTGA	N_{14}	GGGTAT	N_9	TA*G
gtaB (52)	ATGTGTAA	N_{14}	GGGTAA	N_9	TA*G
gsiB (32)	TTGTTTAA	N_{13}	GGGAAT	N_9	C*A*A
gspA (2)	GTGTTTAT	N_{12}	GGGTAT	N_9	TA*C
katE	CAGTTTAT	N_{14}	GGGTAA	N_9	TA*G
Consensus	NNGTTTAA	N_{14}	GGGTAT	N_9	A

[a] Boldface type indicates bases identical to the consensus. Underlining marks the five bases in the proposed −35 and −10 regions that have been shown to be very important for promoter activity in vitro and in vivo (43, 51). Asterisks denote the transcription start sites.

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