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The *Bacillus subtilis sigL* gene encodes an equivalent of σ^{54} from Gram-negative bacteria

(−12, −24 promoters/levanase operon/amino acid catabolism)

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ABSTRACT **The levanase operon in *Bacillus subtilis* is expressed from a −12, −24 promoter and transcription is stimulated by the regulator LevR, which contains a domain homologous with the central domain of the NifA and NtrC family of regulators. We isolated mutants defective in the expression of the levanase operon. These strains contain mutations that define a gene, called *sigL*, located between *cysB* and *sacB* on the genetic map. The *sigL* gene was cloned and sequenced. It encodes a polypeptide containing 436 residues with a molecular weight of 49,644. The amino acid sequence of SigL is homologous with all σ^{54} factors from Gram-negative bacteria, including *Rhizobium meliloti* (32% identity) and *Klebsiella pneumoniae* (30% identity). *B. subtilis sigL* mutants have a pleiotropic phenotype: (*i*) the transcription of the levanase operon is strongly reduced and (*ii*) in minimal medium lacking ammonia, *sigL* mutants cannot grow when arginine, ornithine, isoleucine, or valine is the sole nitrogen source. These results indicate that the *sigL* gene encodes an equivalent of the σ^{54} factor in *B. subtilis*, to our knowledge, the first of this type to be identified in Gram-positive bacteria.**

In Gram-negative bacteria, an alternative σ factor, σ^{54}, allows transcription of genes involved in the assimilation of nitrogen sources—e.g., the gene encoding glutamine synthetase and the genes involved in the transport of amino acids or required for nitrogen fixation (for reviews, see refs. 1–3). In addition, other physiological functions such as catabolism of toluene and xylenes in *Pseudomonas putida*, the formate degradative pathway, and pilin formation are also controlled by σ^{54}. The promoters of these genes are transcribed by RNA polymerase containing σ^{54} and have common features: (*i*) they are devoid of the typical −10, −35 sequences recognized by the major σ factor, but they have strongly conserved −12, −24 regions; (*ii*) they require a positive regulatory protein such as NifA or NtrC that interacts with upstream activating sequences (UAS) to stimulate the isomerization of close complexes between RNA polymerase and the promoter to open complexes.

We have previously shown that the levanase operon of *Bacillus subtilis* is transcribed from a −12, −24 promoter, to our knowledge, the first of this type described in a Gram-positive organism. In addition, the expression of the operon requires a specific regulatory protein, LevR, which contains a 200-amino acid domain similar to the central domains of NifA and NtrC. DNA sequences located upstream, at position −130 with respect to the transcription start site, are also necessary for full expression of the operon. We have shown that in *Escherichia coli* the expression of this operon is strongly dependent upon σ^{54}. These results suggest therefore that an equivalent of σ^{54} exists in *B. subtilis* (4). σ^{54}-like factors have not previously been identified in Gram-positive bacteria.

In this work, we identified and characterized a gene called *sigL* that probably corresponds to the structural gene of the σ^{54} of *B. subtilis*. Strains containing a *sigL* null mutation have a pleiotropic phenotype: the expression of the levanase operon is strongly reduced and the mutants are unable to grow in minimal medium containing only arginine, ornithine, valine, or isoleucine as nitrogen sources. The sequence of the *sigL* gene was determined.† The deduced amino acid sequence is very similar to σ^{54} proteins from Gram-negative bacteria.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* TG1 has been described (5). The following strains of *B. subtilis* were used in this study. BG4100 (*sacB::cat trpC2*), obtained from D. Henner (Genentech), contains a *sacB::cat* insertion mutation disrupting the *sacB* gene encoding levansucrase with a chloramphenicol resistance determinant (*cat*). QB169 (*sacL8 trpC2*) contains a mutation in the *levR* gene (called *sacL8*) leading to constitutive levanase synthesis. QB4106 (*cysB3 hisA1 trpC2*) was constructed by transformation. QB5501 was constructed by introducing a *levD–lacZ* fusion into QB169 as described below. QB5505 (*sigL::aphA3 trpC2*) contains a kanamycin-resistant determinant (6) disrupting the *sigL* gene as described below. QB5501 was treated with ethyl methanesulfonate (7) and white mutants were isolated. One such mutant was taken and designated QB5503. A *recE::cat* insertion mutation (8) was introduced into strain QB5503 by transformation, giving strain QB5504.

Medium, Transformation, and Selection of Recombinants. The minimal medium used contained 50 mM glucose, 100 mM potassium phosphate (pH 7.0), 0.5 mM $MgSO_4$, 0.01 mM $MnSO_4$, 0.02 mM $FeCl_3$, 50 μg of tryptophan per ml, and 20 mM nitrogen source as indicated. *E. coli* was transformed as described (9) and transformants were selected on Luria broth plates containing ampicillin (100 μg/ml). Transformation of *B. subtilis* was as described (10), and transformants were selected on SPem plates (11) containing erythromycin (25 μg/ml), SPcm plates containing chloramphenicol (5 μg/ml), or SPkm plates containing kanamycin (5 μg/ml).

Transduction. PBS1 transduction experiments were carried out as described (7, 12).

Plasmids. pHT305 is a derivative of pHT3101 (13) containing an origin of replication from a *Bacillus thuringiensis* resident plasmid cloned in pUC19 (D. Lereclus, personal communication). pAC7 is a derivative of plasmid pAC2 (4) constructed by replacing the *cat* gene with the *aphA3* gene (14). pAC7 contains a promoterless *lacZ* gene between two

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Abbreviation: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M73443).

fragments of the *B. subtilis amyE* gene. Derivatives of this plasmid cannot replicate in *B. subtilis* but can integrate into the chromosome via homologous recombination at the *amyE* locus. A translational gene fusion of *levD* to *lacZ* was constructed in pAC7 such that the resulting gene encoded the first 54 amino acids of *levD* and all except the first 8 amino acids of *lacZ* as follows. A *Pst* I–*Eco*RV DNA fragment containing *levR* (*sacL8*) and the promoter of the levanase operon was purified from plasmid pRL3 (4), treated with T4 DNA polymerase, and cloned into the *Sma* I site of pAC7, giving pRL8. QB169 was transformed with linearized pRL8, giving QB5501.

Plasmids pSIG2, pSIG3, and pSIG5 were constructed by deleting the *Sst* I, *Eco*RV, or *Pst* I restriction fragments, respectively, from pSIG1 as shown in Fig. 1. pSIG1 is a plasmid isolated from a gene bank of *B. subtilis* DNA (15) constructed in the shuttle vector pMK4 (16). The *Sph* I–*Sal* I fragment of pSIG1 was purified and cloned between the *Sph* I and *Sal* I restriction sites of the polylinker of pHT305, giving pSIG4. The *Sph* I–*Pst* I fragment of pSIG1 was purified and made blunt using T4 DNA polymerase. This DNA fragment was cloned into the *Sma* I site of pHT305, giving pSIG7. A 1.5-kilobase (kb) *Cla* I fragment containing the *aphA3* gene was made blunt using the Klenow fragment of DNA polymerase I and cloned between the two *Eco*RV sites of pSIG5, giving pSIG6 (not shown). Strain 168 was transformed with pSIG6. One kanamycin-resistant, chloramphenicol-sensitive clone, called QB5505, contains the kanamycin cassette integrated into the *sigL* gene by a double crossover event.

β-Galactosidase Assays. *B. subtilis* cells containing *lacZ* fusions were grown at 37°C in Luria broth supplemented with tryptophan (50 μg/ml). β-Galactosidase assays were carried out as described by Miller (17).

DNA Manipulation. DNA sequences were determined by the dideoxynucleotide chain-termination method with single-strand M13 phages as templates (18) and modified T7 DNA polymerase (Sequenase, United States Biochemical). The nucleotide sequence of the second strand was determined by using a series of synthetic oligonucleotides priming at intervals of ≈200 nucleotides.

RESULTS

Isolation of Mutants Defective in the Expression of the Levanase Operon of *B. subtilis*. The promoter proximal four genes of the levanase operon, *levD, levE, levF*, and *levG*, are involved in a fructose-phosphotransferase system and the distal gene, *sacC*, encodes levanase (19, 20). We have previously shown that the levanase operon promoter is positively controlled by the *levR* gene, which is located just upstream of the operon (4).

We have designed a screening procedure to isolate mutants defective in regulatory genes affecting the expression of the levanase operon. Mutants affected either in the structural gene of the σ^{54} of *B. subtilis* or in the *levR* gene would be expected to be found by this procedure. To avoid the selection of *levR* mutants, a merodiploid strain was constructed. This strain (QB5501) contains two copies of the *levR* constitutive allele (*sacL8*), one copy located at the original levanase locus and the other one located upstream from a *levD–lacZ* translational fusion introduced at the *amyE* locus. In this fusion strain, the *lacZ* gene is expressed from the promoter of the levanase operon and *lacZ* and *sacC* were indeed constitutively expressed. QB5501 displayed a dark blue phenotype when plated on 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) plates. A collection of mutants was obtained by ethyl methanesulfonate mutagenesis of QB5501. A total of 16 white mutants was independently obtained. Among them, two classes were distinguished on the basis of their phenotypes. In 10 mutants, *sacC* expression was constitutive. They were probably therefore affected in *lacZ* expression and hence were discarded. Six mutants in which *lacZ* and *sacC* expression was strongly reduced were kept for further study. β-Galactosidase synthesis was assayed in a culture of one of these mutants, QB5503. In this mutant, *lacZ* expression was abolished (6 units/mg of protein) as compared to the parental strain QB5501 (820 units/mg of protein).

The gene affected in QB5503 was cloned by complementation as follows. QB5503 was transformed with a *B. subtilis* gene bank (15) in a *B. subtilis/E. coli* shuttle vector (16), and transformants were screened for blue colonies on X-Gal plates. Five blue colonies were observed among about 10,000 transformants. Plasmids from these blue clones were transferred into *E. coli* for characterization. A restriction map was established for one plasmid called pSIG1 (Fig. 1). Plasmid pSIG1 was reintroduced by transformation into the 6 ethyl methanesulfonate-induced *B. subtilis* mutants. Among the transformants of each mutant isolated on SPcm/X-Gal plates, 80% had a dark blue phenotype, indicating complementation of *lacZ* expression. The remaining 20% white

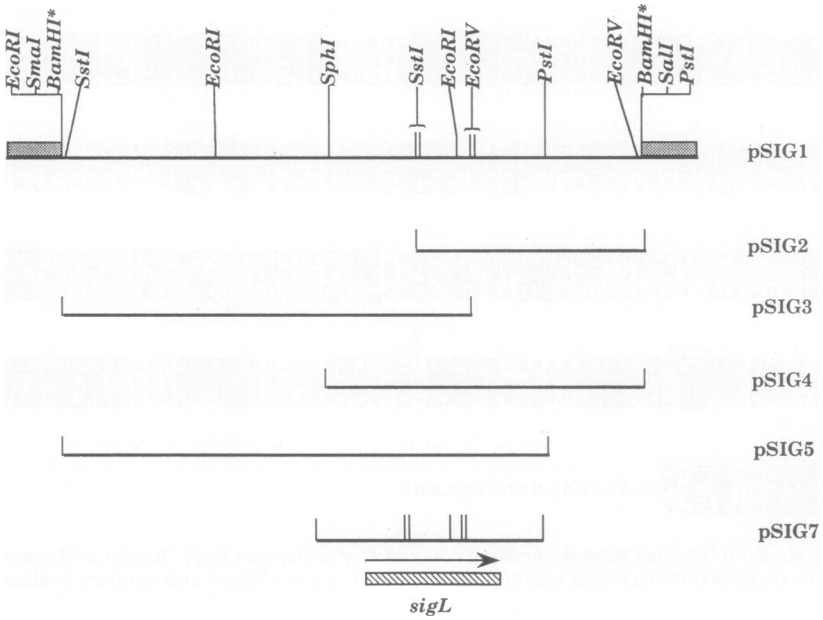

FIG. 1. Simplified restriction map of plasmids containing the cloned fragment used in this work. pSIG1, pSIG2, pSIG3, and pSIG5 are pMK4 derivatives. pSIG4 and pSIG7 are pHT305 derivatives. *Bam*HI* indicates that the *Bam*HI site was lost as a consequence of construction. In pSIG1, boxes correspond to the pMK4 vector.

transformants were probably due to recombination between the gene cloned on the plasmid and its defective homologue on the chromosome, as shown below. This result strongly suggests that all 6 mutants are affected in the same gene, which was designated *sigL*.

Genetic Fine Structure Analysis of the *sigL* Locus. To locate precisely the gene isolated in plasmid pSIG1, deleted derivatives of this plasmid were constructed (see *Materials and Methods*) and tested for complementation of the QB5503 *sigL* mutant. Plasmids pSIG2, pSIG3, and pSIG5 were derived from pSIG1 by eliminating the *Sst* I, *Eco*RV, or *Pst* I DNA fragments, respectively (Fig. 1). Plasmids pSIG4 and pSIG7 were obtained by cloning the *Sph* I–*Sal* I or the *Sph* I–*Pst* I restriction fragment into the polylinker of the replicative plasmid pHT305. Plasmids pSIG1, pSIG4, pSIG5, and pSIG7 were each used to transform QB5503. In each case, 80–90% of the colonies on X-Gal plates were blue, indicating complementation of the *sigL* mutation. All four plasmids restored β-galactosidase and levanase activity. In contrast, the introduction of pSIG2 or pSIG3 into QB5503 gave 80% white colonies on X-Gal plates. This persistent heterogeneity could be due to recombination between the cloned fragment and chromosomal DNA. These experiments led to the conclusion that the *sigL* gene overlaps the two *Sst* I and the two *Eco*RV restriction sites present in pSIG1. To confirm the complementation data obtained with pSIG1 and deleted derivatives, a *recE* null mutation was introduced by transformation into QB5503, giving QB5504 (see *Materials and Methods*). Transformants obtained with pSIG7 were all blue on SPem/X-Gal plates, indicating complementation of *lacZ* gene expression. Specific activities of β-galactosidase were determined for strain QB5504 (*recE::cat sigL*) carrying either pSIG7 or the pHT305 vector as a control and were 775 units/mg of protein and 5 units/mg of protein, respectively. The level of *lacZ* gene expression obtained with pSIG7 was almost identical to that obtained with the parental *sigL*$^+$ strain, QB5501, grown in the same conditions (820 units/mg of protein). The expression of *sacC* was also constitutive in QB5504 carrying pSIG7, as revealed by the plate test for sucrose hydrolysis.

These results indicate that the *sigL* gene, or at least a functional part of it, is located between the *Sph* I and the *Pst* I restriction sites of plasmid pSIG1 (see Fig. 1).

Nucleotide Sequence of the *sigL* Gene. The DNA sequence of a 2.3-kb fragment of pSIG7 was determined on both strands (see *Materials and Methods*). The sequenced region extends from 600 base pairs (bp) upstream from the upstream *Sst* I restriction site to 1000 bp downstream from the downstream *Eco*RV restriction site. An open reading frame was found, containing a total of 475 codons ending with a TAA. The most likely coding region is preceded by a putative ribosome binding site (AAAGGGGAGTGA, ΔG = −56.8 kJ/mol) and begins with an ATG codon. The open reading frame extends 117 nucleotides upstream from the proposed ATG start codon. Since no other start codon preceded by a ribosome binding site at a correct distance was present upstream, this ATG is presumably the translational initiation codon of the SigL protein. The SigL protein would therefore be composed of 436 residues with a deduced molecular weight of 49,644.

A computer search for similarities with other proteins revealed that the SigL polypeptide is similar to all σ^{54} factors from Gram-negative bacteria. Fig. 2 presents an alignment of the *B. subtilis* SigL polypeptide with σ^{54} of *Rhizobium meliloti* (32% identity) (21) and *Klebsiella pneumoniae* (30% identity) (22).

As in other σ^{54} factors, two conserved regions are present: an amino-terminal region (region I) composed of 50–80 residues and a long region extending approximately from position 100 to the end of the protein (region III). Between these two well-conserved regions, a short region (region II) of variable length shows little similarity between any pair of σ^{54} proteins. It has been already proposed that region II does not contain any structure critical for function (23, 24).

A potential DNA binding domain has also been identified in the carboxyl-terminal part of the region III of the σ^{54} factors by comparison with other DNA binding helix–turn–helix motifs (23). This sequence, between residues 320 and

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Bs   1   MDMKLQQVQVLKPQLTQELRQAITLLGYHSAELAEYLDELSLENPLIERKETDTPPLSYHKT----------------
Rm   1 MALSASLHLRQSQSLVMTPQLMQSIQLLQMNHLELSHFLAQEVEKNPLLEVQPADEPTISDREDAGPHPAETGGETDEAA
Kp   1   MKQGLQLRLSQQLAMTPQLQQAIRLLQLSTLELQQELQQALDSNPLLEQTDLHDEVETKEAEDRESLDTVDALEQKEM

Bs  63 ------------------------------------------------------NKNRMNAQEAGLQLSNPQKTLQDALKQQS
Rm  81 GQSDLYDSAMSRSGERLSEGLDADFANVFPDDTAPQRADAPELLGQWKSMPGAGDAEGYDLDDF-VGGRKTLRETLAEQL
Kp  79 ------------------------------PEELPLDASWDEIYTAGTPSGNGVDYQDDELPVYQGETTQSLQDYLMWQV

Bs  92 LDMNLTNTEKKIFNYLIHSLDSNGYLEEDIEEAARRLSVSAKEAEAVLAKLQSLE---EAGIGARSLQECILLQLQRLPN
Rm 160 PFALSAVSDRLIARYFLDQLDDAGYLHADLAETAETLGAAGEDVARVLHVLQQFD---EPGVFARTLGECLAIQLRARN-
Kp 129 ELTPFTDTDRAIATSIVDAVDDTGYLTISVEDIVESIGDDEIGLEEVEAVLKRIQRFDEVGVAAKDLRDCLLVQLSQFAK

Bs 169 ---RNEQAEMLVSAHFDAFAQKKWKTLSVETGIPLHTIQDISDDIAALHPRPGLLFARPSQDVYIEPDIFITVK-NGHIA
Rm 236 ---RLDPAMEALVANLELLARRDFASLKKICGVDEEDLIDMLAEIRKLDPKPGTSFETGVFE-AIIPDVVVRAAPDCGWL
Kp 209 ETPWIEEARLIISDHLDLLANHDFRSLMRVTRLKEEVLKEAVNLIQSLDPRPGQSIQTGEPE-YVIPD-VLVRKVNDRWV

Bs 245 AELNTRSFPEIDLHPQY--RTLLSSGSCQDTVSYLSAKYQEWRWLSRALRQRKQTITRIINELITRQKDFFLKGRSAMKP
Rm 312 VELNPDALPRVLVNHDYFTEISRSSRKNSGEQAFLNECLQNANWLTRSLDQRARTIMKVASEIVRQQDAFLIHGVGHLRP
Kp 287 VELNSDSLPRLKINQQY-AAMGNSTRNDADGQ-FIRSNLQEARWLIKSLESRNDTLLRVSRCIVEQQQAFFEQGEEFMKP

Bs 323 LTLREVADCLSLHESTVSRAIKGKTIQTPYGLFEMKLFFSAKAEASGDGDA-SNYAVKTHLENLINQEDKTKPLSDQKLV
Rm 392 LNLRIVADAIKMHESTVSRVTSNKYMLTPRGLFELKYFFTVSIGSAENGDAHSAESVRHRIRTMINQESADAVLSDDDIV
Kp 365 MVLADIAQAVEMHESTISRVTTQKYLHSPRGIFELKYFFSSHVNTEGGGEA-SSTAIRALVKKLIAAENPAKPLSDSKLT

Bs 402 DLLYEQHGIQISRRTVAKYRDQMNIPSSAARKRYK
Rm 472 DVLK-QAGVDLARRTVAKYREAMSIPSSVQRRREKRALPRPRDSERCRQAASA
Kp 444 TMLSDQ-GIMVARRTVAKYRESLSIPPSNQRKQLV
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FIG. 2. Comparison of *B. subtilis* (Bs) SigL with similar σ^{54} factors from *R. meliloti* (Rm) and *K. pneumoniae* (Kp). Amino acid sequences of the polypeptides were aligned by introducing gaps (hyphens) to maximize identity. Identical residues are boxed and numbers indicate the positions of the residues in each polypeptide.

345, is well conserved in σ^{54} of *B. subtilis*. The Dodd and Egan method was used for the prediction of potential helix–turn–helix motifs. A standard deviation (SD) score of 6.38 SD was obtained, indicating that this region has a 100% probability of being a helix–turn–helix motif according to this method of calculation (25).

Another strongly conserved sequence (ARRTVAKYRE) has been found in all characterized σ^{54} factors (24). Eight of 10 residues are also present in the corresponding region of the *B. subtilis* σ^{54} (position 414–421). The function of this sequence is as yet unknown.

The amino-terminal region that is well conserved in other σ^{54} factors is also conserved in the σ^{54} of *B. subtilis*. The first 21 amino acids of region I contain 6 glutamine residues (Fig. 2) and the regions 31–53 and 112–137 contain a combined total of 16 acidic residues. Moreover, there are two conserved motifs of 26 amino acids, each in the amino-terminal region, which include two leucine-rich heptad hydrophobic repeats that could form a leucine zipper structure (26) (Fig. 3).

Thus, the deduced SigL protein has all of the characteristic regions found in all σ^{54} factors of Gram-negative bacteria.

Disruption of the *sigL* Gene and Phenotypes of *sigL* Mutants. A kanamycin cassette (*aphA3*) was introduced into pSIG5 between the two *Eco*RV restriction sites, resulting in pSIG6. This *sigL* null mutation was reintroduced into the chromosome of the *sacL8* constitutive strain QB169 using pSIG6 by a double crossover event. In this strain, the expression of the *sacC* gene was completely abolished. This result indicates that the *sigL* gene encodes a positive regulatory factor necessary for levanase expression.

In Gram-negative bacteria, the σ^{54} has physiologically diverse roles and strains that lack this factor have pleiotropic phenotypes. Nevertheless, it is not essential for viability under all conditions tested (2).

To analyze the functional role of σ^{54} of *B. subtilis*, we used strain QB5505, which contains an *aphA3* insertion inactivating the *sigL* gene. This mutant was tested for growth in glucose minimal media, each containing only one nitrogen source such as NH_4^+, aminobutyrate, urea, or nitrate. The growth of QB5505 on these nitrogen sources was indistinguishable from that of the parental wild-type strain (not shown).

E. coli strains lacking σ^{54} are glutamine auxotrophs and cannot utilize arginine as nitrogen source. Thus, individual L-amino acids were tested for their ability to support the growth of *B. subtilis* $sigL^+$ or $sigL^-$ strains in glucose minimal medium. Three classes of amino acids could be distinguished. (*i*) Class I comprises amino acids allowing growth of $sigL^+$ and $sigL^-$ strains: alanine, aspartate, glutamate, asparagine, proline, and glutamine. Threonine and histidine allow slow growth and, again, no difference between growth rates of $sigL^+$ and $sigL^-$ strains was observed (not shown). (*ii*) Class II includes amino acids that, when used as the sole nitrogen source, do not support the growth of either $sigL^+$ or $sigL^-$ strains: cysteine, phenylalanine, tyrosine, glycine, lysine, leucine, methionine, serine, and tryptophan. (*iii*) Class III includes amino acids that support the growth of $sigL^+$ strains but not that of $sigL^-$ strains: arginine, ornithine, valine, and isoleucine, whose utilization is therefore σ^{54} dependent (Table 1).

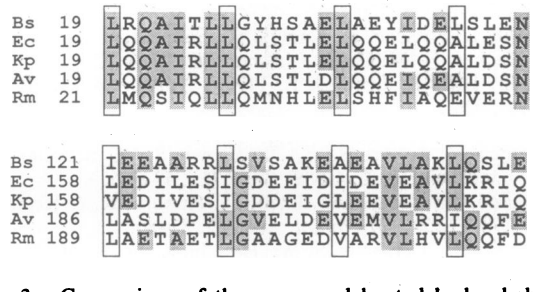

FIG. 3. Comparison of the conserved heptad hydrophobic repeats in σ^{54} factors. These regions could form leucine zipper structures according to Sasse-Dwight and Gralla (26). Identical residues are boxed and numbers indicate the positions of the residues in the proteins from *B. subtilis* (Bs), *E. coli* (Ec), *K. pneumoniae* (Kp), *Azotobacter vinelandii* (Av), and *R. meliloti* (Rm).

Table 1. Doubling time of 168 and QB5505 (*sigL*) strains grown in minimal medium containing various nitrogen sources

Nitrogen source	Doubling time, hr	
	168 ($sigL^+$)	QB5505 ($sigL^-$)
Arginine	0.8	≈8
Ornithine	0.85	≈8
Isoleucine	3.0	>10
Valine	2.0	>10
Glutamine	0.8	0.8
Ammonium sulfate	1.1	1.1

Location of the *sigL* Gene on the *B. subtilis* Genetic Map. The *sigL* gene was located on the genetic map by transduction crosses using bacteriophage PBS1. A PBS1 lysate prepared from strain QB5505 containing the kanamycin cassette disrupting the *sigL* gene was used for transduction of strain QB4106. After selection for kanamycin resistance, linkage could be shown between the *sigL::aphA3*, *cysB3*, and *hisA1* markers. Introduction of the *sigL::aphA3* cassette by transformation into the genetic background of strain QB4106 (*cysB3 hisA1 trpC2*) led to the construction of strain QB5506 (*sigL::aphA3 cysB3 hisA1 trpC2*). This strain was used as the recipient in a four-factor transductional cross using a PBS1 lysate from strain BG4100, which contains a chloramphenicol cassette in the *sacB* gene. This cross allowed us to map these genetic markers in the following order: *cysB3*, *sigL*, *sacB*, *hisA1* (Fig. 4).

DISCUSSION

The amino acid sequence deduced from the *sigL* DNA sequence of *B. subtilis* is similar to the sequences of nine σ^{54} factors from Gram-negative bacteria (24, 26). The characteristic domain organization of σ^{54} factors is conserved in the *B. subtilis* SigL: a glutamine-rich and an acidic-rich amino-terminal region, a putative leucine zipper, and a helix–turn–helix motif.

The promoters controlled by σ^{54} have conserved residues around positions −12 and −24 with respect to the initiation site. Sasse-Dwight and Gralla (26) suggest that the σ^{54} factor contacts the −12 and the −24 promoter regions. Two leucine-rich heptad hydrophobic repeats could dimerize to form a leucine zipper that makes contact with the −12 region of the promoter. The helix–turn–helix motif may help to bind the polymerase to the DNA through contacts involving the −24 region (26). In the *E. coli* σ^{54} factor, two acidic regions are believed to be involved in the melting step during the initi-

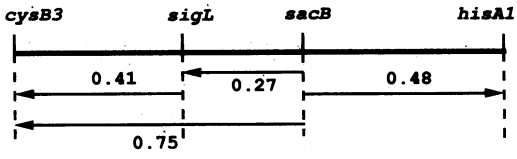

FIG. 4. Genetic map of the *B. subtilis* chromosomal region containing the *sigL* gene. This map was obtained from three-factor and four-factor crosses by PBS1 transduction. The numbers represent values of 1 − the cotransduction frequency. The arrows point from the selected to the nonselected markers.

ation of transcription. The formation of the open complex also specifically requires the product of another regulatory protein in addition to σ^{54}: NtrC for the glutamine synthetase gene, DctD for dicarboxylate transport in *R. meliloti*, and *Rhizobium leguminosarum*, XylR for catabolism of toluene and xylenes in *P. putida*, NifA for nitrogen fixation genes in *K. pneumoniae*, and LevR in the case of the levanase operon of *B. subtilis* (2–4).

The σ^{54} factors of Gram-negative bacteria and its equivalent in *B. subtilis* are all positive regulatory factors for degradative enzymes—e.g., levanase of *B. subtilis* (this paper), the enzymes involved in arginine catabolism in *E. coli* and *B. subtilis*, and the enzymes involved in xylene and toluene catabolism in *P. putida* (1, 2). An important difference, however, is that *B. subtilis sigL* null mutants are not glutamine auxotrophs unlike Gram-negative σ^{54} mutants. σ^{54} is required by Gram-negative bacteria for the expression of glutamine synthetase, a key enzyme, that has a dual role. (*i*) It allows assimilation of ammonium ions, the preferred nitrogen source for growth of enteric bacteria, in a defined minimal medium with glucose as the carbon source. It should be noted that amino acids (such as glutamine, arginine, and maybe others) rather than ammonium are the preferred nitrogen sources in *B. subtilis* since they allow faster growth rates (this paper). (*ii*) Glutamine synthetase encoded by the *glnA* gene, as well as σ^{54}, is part of a central control system in Gram-negative bacteria affecting transcription of nitrogen-regulated operons. Two additional genes of the *glnALG* operon are involved in this regulatory system: *glnL* and *glnG*, encoding the NtrB and NtrC proteins, respectively. The NtrB sensor kinase responds to the nitrogen status of the cell and modulates the activity of the NtrC response regulator. In conditions of limiting nitrogen, NtrB activates NtrC by phosphorylating it, whereas in nitrogen excess, NtrC is dephosphorylated and consequently inactivated by NtrB (1).

In *B. subtilis*, no candidate genes for NtrB or NtrC equivalents have been found in the vicinity of the structural gene *glnA*, encoding glutamine synthetase (27, 28). Regulation of glutamine synthetase expression is apparently rather different from that observed in Gram-negative bacteria. Synthesis of glutamine is regulated by two proteins of the *glnRA* operon. The product of the *glnA* gene, presumably glutamine synthetase itself, is necessary for negative regulation of *glnRA* transcription. The *glnR* gene codes for a 16-kDa repressor, which possesses a putative helix–turn–helix motif. A possible model for *glnRA* regulation is that, under conditions of nitrogen excess, glutamine synthetase interacts with or modifies the *glnR* product, causing it to bind to the *glnRA* promoter region and repress transcription (29).

Another property of the central nitrogen sensing system in Gram-negative bacteria is that it leads to a decrease of the catalytic activity of glutamine synthetase by adenylylating the enzyme in conditions of nitrogen excess (1). In *B. subtilis*, there is no evidence for adenylylation or any other inactivation mechanism of the glutamine synthetase catalytical activity (30).

Ammonium-generating degradative enzymes are regulated by four distinct systems in *B. subtilis*: (*i*) the system mediating amino acid repression of histidine and proline degradative enzymes (31); (*ii*) the system controlling the expression of glutamine synthetase, which is dependent on the GlnA and GlnR proteins (32); (*iii*) the system controlling the expression of asparaginase and urease, which could partially overlap with system *ii*, since GlnA, but not GlnR, is involved in this regulation (32); (*iv*) σ^{54}-dependent regulation (this paper). It would be interesting to know whether σ^{54}-mediated regulation and the other systems are interrelated.

It seems therefore that *B. subtilis* does not possess the same adaptative system as that found in enteric bacteria. However, the nitrogen sensing system of Gram-negative bacteria may not be needed in *B. subtilis*, since this bacterium can sporulate when growth slows down or is prevented due to nitrogen deprivation.

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