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The transcriptional regulator LevR of *Bacillus subtilis* has domains homologous to both σ^{54}- and phosphotransferase system-dependent regulators

(−12, −24 promoters/NifA/antiterminators/upstream activating sequence)

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ABSTRACT **The regulatory gene *levR* of the levanase operon of *Bacillus subtilis* was cloned and sequenced. It encodes a polypeptide of M_r 106,064 with two domains homologous to members of two families of bacterial activators. One domain in LevR is homologous with one region of bacterial regulators including SacT and SacY of *B. subtilis* and BglG from *Escherichia coli*. Another domain of LevR is homologous to one part of the central domain of NifA and NtrC, which control nitrogen assimilation in Gram-negative bacteria. The levanase promoter contains two regions almost identical to the −12, −24 consensus regions present in σ^{54}-dependent promoters. The expression of the levanase operon in *E. coli* was strongly dependent on σ^{54}. Taken together, these results suggest that the operon is expressed from a −12, −24 promoter regulated by a σ^{54}-like-dependent system in *B. subtilis*.**

In *Bacillus subtilis*, the expression of the levanase operon is inducible by fructose and is subject to catabolite repression (1, 2). A fructose-inducible promoter has been characterized 2.7 kilobases (kb) upstream from the *sacC* gene, which encodes levanase. *sacC* is the distal gene of an operon containing five genes: *levD, levE, levF, levG*, and *sacC* (3). The first four gene products are involved in a fructose-phosphotransferase system (fructose-PTS) in *B. subtilis* and share homology with the mannose-PTS of *Escherichia coli* (3). *sacL* mutants that constitutively express the levanase operon have been isolated (1). Three of the corresponding mutations have been located by DNA sequencing within the two most upstream genes of the levanase operon. The analysis of these constitutive mutations led to the conclusion that *levD* and *levE* gene products are involved in a fructose-PTS and are also negative regulators of the expression of the levanase operon. A specific component of the PTS is involved in induction of the *bgl* operon, which allows the utilization of β-glucosides in *E. coli* (4, 5). By analogy with the *bgl* system, the following model of regulation was proposed: in the presence of fructose LevD, LevE, LevF, and LevG polypeptides with the general proteins of the PTS make up a phosphotransferase cascade, leading to the transport and phosphorylation of fructose. In the absence of fructose, the phosphate group is transferred probably via the *levD* and *levE* gene products to the product of a regulatory gene not yet identified, thereby abolishing its activity (3).

In this work, we have cloned the upstream region of the levanase operon and identified a positive regulatory gene called *levR*, which controls the expression of the operon.† The deduced LevR polypeptide has a molecular mass of 106 kDa. It contains two domains. Domain A shares similarity with the central domain of NifA and NtrC, two activator proteins controlling assimilation and fixation of nitrogen in several Gram-negative bacteria including *Klebsiella pneumoniae* and *Rhizobium meliloti* (6, 7). Domain B is similar to SacT and SacY, two regulatory proteins of the sucrose regulon in *B. subtilis* (8, 9), and to BglG, the regulatory protein of the *bgl* system in *E. coli* (10, 11). The levanase promoter contains two regions almost identical to the −12, −24 consensus regions present in σ^{54}-dependent promoters (12). Moreover, it is shown that, in *E. coli*, expression of the levanase operon requires the presence of both *levR* and *ntrA* gene products.

MATERIALS AND METHODS

Bacterial Strains. *E. coli* TG1 (13) was used as a host for pHV1431d derivatives (14, 15), for pHT3101 derivatives (16), for pAC2 derivatives (see below), and for the sequencing vectors mp18 and mp19 (17). pHV1431d contains the pAMβ1 origin of replication (14). pHT3101 contains an origin of replication from a *Bacillus thuringiensis* resident plasmid (16). pAC2 contains the pBR322 origin of replication (18). *E. coli* ET8000 *ntrA*$^+$ and ET8045 *ntrA*::Tn*10* (19) were used to test the expression of the levanase operon. *B. subtilis* 168 *trpC2* and QB169 *trpC2 sacL8* (1) were used as recipient strains during the construction of pRL2 and pRL3 plasmids. *sacL*$^+$ and *sacL8* alleles of *levR* on multiple copy plasmids were introduced into, and maintained in, *B. subtilis* 1A510 *recE4 leuA8 arg15 thrA5 stp* (20). Strain QB5500 was constructed as follows. QB5030 *trpC2 sacC–lacZ erm* (3) was transformed with linearized pJC30 plasmid. The kanamycin cassette was introduced into the chromosome by homologous recombination. One kanamycin-resistant (*kan*^{R}) chloramphenicol-sensitive (*cm*^{S}) transformant was purified and characterized as QB5500 *levR*::*aphA3 trpC2 sacC–lacZ erm*. QB5038 *levD*::*aphA3 trpC2* was constructed in the same way except that linearized pJC23 was used to transform *B. subtilis* 168. One *kan*^{R} *cm*^{S} transformant (QB5038) was isolated.

Plasmids. The plasmids used in this work are described in Fig. 1. pJC30 was constructed as follows. A 1.5-kb *Cla* I restriction fragment containing the *aphA3* gene (21) encoding kanamycin resistance was purified from plasmid pKa (8). This DNA fragment was cloned into the single *Cla* I site of pJC6 (2, 3). pJC23 was constructed by inserting the 1.5-kb *aphA3* DNA fragment into the *Eco*RV site of pJC6. During this construction, the *Cla* I restriction sites were made blunt by using the Klenow fragment of DNA polymerase I. To construct a *B. subtilis* gene bank, *Pst* I-linearized pHV1431d plasmid DNA and *Pst* I-digested chromosomal DNA of

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Abbreviations: PTS, phosphotransferase system; UAS, upstream activating sequence; ORF, open reading frame.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60105).

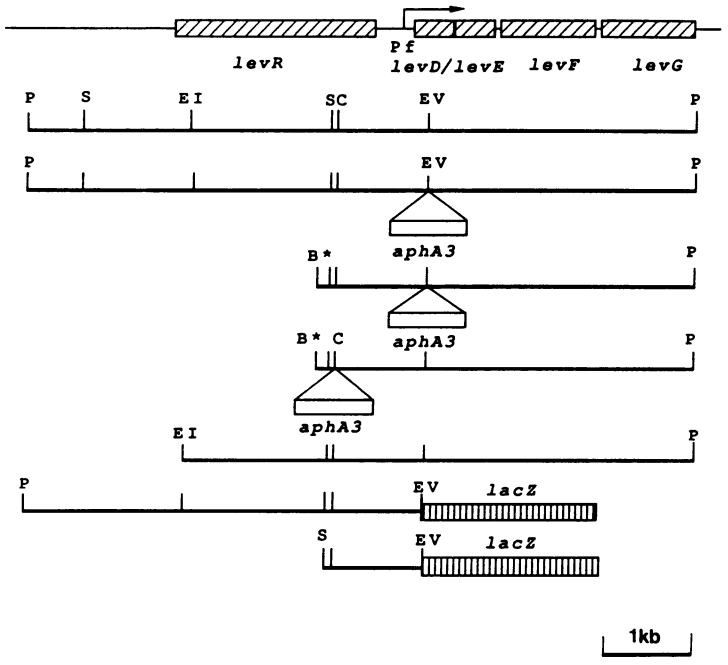

FIG. 1. Simplified restriction maps of the cloned DNA fragments used in this work. The beginning of the *sacC* gene is located 127 bp downstream from the *Pst* I site after the *levG* gene (3). pRL1, pRL2, pRL3, and pRL4 are *E. coli/B. subtilis* replicons. pRL2 and pRL3 contain the wild-type allele and the constitutive allele (*sacL8*) of *levR*, respectively. *levD* and *levR* are interrupted in plasmid pRL1, pJC23, and pJC30 by an *aphA3* cassette. pJC23, pJC30, pRL5, pRL6, and pRL7 are *E. coli* replicons. Pf, fructose-inducible promoter. B*, *Bam*HI restriction site was not regenerated during the cloning of the partial *Sau*3A fragment. P, *Pst* I; S, *Stu* I; EI, *Eco*RI; C, *Cla* I; EV, *Eco*RV.

QB5038 were ligated at a high concentration (50–100 μg/ml) and used for direct transformation of competent cells of *B. subtilis* 1A510 (Rec$^-$). One *kan*^{R} clone was purified and the plasmid DNA was extracted. This plasmid was called pRL0 (data not shown). The 7.5-kb *Pst* I fragment cloned in pRL0 was transferred into the *Pst* I site of the plasmid pHT3101 giving pRL1. pRL2 (*sacL*$^+$) and pRL3 (*sacL8*) were obtained by *in vivo* recombination in the 168 (Rec$^+$) and QB169 (Rec$^+$) strains, respectively, as described (8). Plasmid pRL4 was constructed as follows. An *Eco*RI restriction site is located in the pUC19 polylinker of plasmid pRL3, downstream from the *levG* gene (Fig. 1). A 4-kb *Eco*RI fragment was purified from pRL3 and cloned into the single *Eco*RI site of pHT3101 to give pRL4. pRL5, pRL6, and pRL7 plasmids were constructed as follows. An 8-base-pair (bp) *Bgl* II linker was cloned into the *Sma* I site of pAF1 (18). Subsequently, the *Eco*RI/*Sac* I fragment of pIS112 (22) was purified and cloned between the *Eco*RI and *Sac* I sites of the resulting plasmid, giving pAC2. A translational gene fusion of the amino-terminal part of *levD* to codon 8 of *lacZ* was constructed in pAC2. *Pst* I/*Eco*RV DNA fragments containing *levR* and the promoter of the levanase operon were purified from pRL2 (*sacL*$^+$) and pRL3 (*sacL8*), treated with T4 DNA polymerase enzyme, and cloned into the *Sma* I site of pAC2, giving pRL6 (*sacL*$^+$) and pRL7 (*sacL8*). A *Stu* I/*Eco*RV fragment containing the promoter of the operon was purified from pRL2 and cloned into the *Sma* I site of pAC2 plasmid, resulting in pRL5.

Transformation and Selection of Recombinants. *E. coli* was transformed as described (2) with selection on Luria broth plates containing ampicillin (100 μg/ml). Transformation of *B. subtilis* was as described (15) and selection was carried out on SP plates (2) containing erythromycin (25 μg/ml) or kanamycin (5 μg/ml).

β-Galactosidase Assays. *E. coli* cells containing *lacZ* fusions were grown at 37°C in M9 medium (23) containing 0.4% glycerol, 0.1% L-glutamine (7 mM), and ampicillin (100 μg/ml). β-Galactosidase assays were carried out as described by Miller (23). *B. subtilis* cells containing *lacZ* fusions were grown in CSK (medium C supplemented with potassium succinate and potassium glutamate) minimal medium (8) containing 0.2% fructose as the inducer when needed and each auxotrophic requirement at 100 μg/ml.

DNA Manipulations. DNA sequences were determined by the dideoxynucleotide chain-termination method with single-strand M13 phages as template (24) 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 that prime at intervals of 200 nucleotides. The *sacL8* mutation was previously mapped between the promoter of the levanase operon and the *Bam*HI* of pJC6 (3). A 642-bp DNA fragment containing the sequence upstream of the operon promoter was amplified by the PCR technique. Two oligonucleotides, 5′-AAAGGATCCAACACAGTTGTGTTAAGCG-3′ centered on the −40 region of the promoter and 5′-GGGAATTCTGGAAGACATTCTAACCACG-3′ corresponding to the *Stu* I restriction site located in *levR*, were used for DNA amplification. The two oligonucleotides include mismatches to the wild-type sequence leading to the creation of *Eco*RI and *Bam*HI restriction sites. The amplified fragment was cloned in mp18 and mp19 vectors. Three independently isolated templates were sequenced for each mutant to identify the errors due to the amplification procedure (25). The DNA sequence located between the *Stu* I and *Bam*HI* sites was obtained as follows. The 2.8-kb *Stu* I fragment from pRL3 (Fig. 1) was isolated and cloned into vector mp18 linearized with *Sma* I. The DNA sequence of the insert was determined on both strands.

RESULTS

Characterization of a Regulator of the Levanase Operon. Two lines of evidence strongly suggested that a regulatory gene located just upstream from the levanase operon plays a role in the operon expression. (*i*) The end of an open reading frame (ORF), at least 600 bp long was previously observed preceding the promoter region (3). Gene disruption experiments were carried out to inactivate the expression of this gene by the introduction of a cassette containing a kanamycin-resistance determinant into the ORF. The construction was introduced into the chromosome of *B. subtilis* QB5030 by homologous recombination via a double crossover event, giving strain QB5500. The QB5030 contains a *sacC–lacZ* transcriptional fusion allowing a convenient assay for levanase expression (2). Strains QB5030 and QB5500 were grown in CSK minimal medium in the presence or absence of 0.2%

fructose as the inducer. β-Galactosidase activities were determined and are presented in Table 1. Disruption of the upstream ORF results in a total loss of inducibility of the levanase operon. It was therefore concluded that the product of this ORF is essential for the expression of the operon. (*ii*) The *sacL8* constitutive mutation was previously mapped in a 900-bp fragment upstream from the promoter of the levanase operon (3). DNA sequencing of this region showed that this mutation is located within the coding sequence of the regulatory gene (see below). These results prompted us to clone the upstream region of the promoter of the levanase operon.

Cloning of the Regulatory Gene. The strategy used to clone the upstream region was to construct a strain containing a selective marker (*aphA3*) inserted in the first gene of the operon (Fig. 1). A kanamycin cassette was introduced into *levD* of strain 168, giving strain QB5038. The close linkage between the upstream ORF and the first gene *levD* was used to clone directly in *B. subtilis* the regulatory gene. Plasmid pRL1 was obtained in this way. A restriction map of pRL1 is shown in Fig. 1. To study the effect of the *sacL8* allele in a $sacL^+$ background, the *sacL8* constitutive allele was cloned *in vivo* by gene conversion starting from plasmid pRL1. The resulting plasmid was called pRL3 (Fig. 1). As a control, a $sacL^+kan^S$ derivative, called pRL2, was obtained from pRL1 in the same way (Fig. 1). pRL2 ($sacL^+$) and pRL3 (*sacL8*) plasmids were introduced by transformation into the 1A510 (Rec^-) strain of *B. subtilis*. A high constitutive level of levanase synthesis (320 units per mg of protein) was observed in the strain containing plasmid pRL3. The level of levanase synthesis induced by 0.2% fructose in 1A510 (Rec^-) strain containing pRL2 is 10 units per mg of protein. This low level of levanase synthesis in the wild-type-induced strain could be a consequence of catabolite repression as observed (1, 2). This indicates that the *sacL8* allele placed on a multiple copy plasmid is functional and dominant over the wild type. To characterize further the regulatory gene on the 7.5-kb *Pst* I fragment, subcloning experiments were performed. The resulting plasmid pRL4 (Fig. 1) was introduced by transformation into the 1A510 strain of *B. subtilis*. Levanase expression was tested on SP plates containing erythromycin (2). Expression was abolished in this case (data not shown). This result shows that sequences located upstream from the *Eco*RI restriction site are required for full expression of the levanase operon.

DNA Sequence of the Upstream Region of the Levanase Operon. The DNA sequence of a 3.0-kb DNA fragment upstream from the levanase operon promoter was determined on both strands by the dideoxynucleotide chain-termination method. The sequenced region extends 150 bp upstream from the *Eco*RI restriction site (Fig. 1). An ORF starting with an ATG codon preceded by a putative ribosome binding site (SD) AAGGA was found. This ORF encodes a polypeptide of 938 residues with a deduced molecular weight of 106,064 (Fig. 2). A region of dyad symmetry followed by a region rich in T residues is found 19 bp after the stop codon. This region could form a stem and loop structure and may be a transcription terminator (see Fig. 4). The nucleotide sequence of the *sacL8* mutation was determined by using chromosomal DNA from strain QB169 (*sacL8*), amplified by the PCR technique. The *sacL8* mutation is located in the *levR* regulatory gene. This mutation corresponds to a C → T transition transforming a CAG codon (Gln-798) into TAG (stop).

Table 1. Effect of disruption of the *levR* gene in *B. subtilis*

Strain	Relevant genotype	β-Galactosidase specific activity: CSK medium	β-Galactosidase specific activity: CSK Fru medium
QB5030	*sacC–lacZ*$^+$	30	420
QB5500	*sacC–lacZ*$^+$*levR::aphA3*	10	2

Cultures were grown at 37°C in medium C supplemented with potassium glutamate and potassium succinate (CSK) with or without 0.2% fructose (Fru) as the inducer. β-Galactosidase specific activities were determined in extracts prepared from exponentially growing cells. The values mentioned are the mean values of three independent measurements and are expressed as Miller units per mg of protein.

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MIDVRRIDKIYHQLKHNFHDSTLDHLLKIQGNSAKEIAEQLKMERSNVSF  50
ELNNLVRSKKVIKIKTFPVRYIPVEIAEKLFNKKWDTEMMEVKDLQAFSG 100
NSKQNHQHISTNPLELMIGAKGSLKKAISQAKAAVFYPPNGLHMLLLGPT 150
GSGKSLFANRIYQFAIYSDILKAGAPFITFNCADYYNNPQLLLSQLFGHK 200
KGSFTGAAEDKAGLVEQANGGILFMDEIHRLPPEGQEMLFYFIDSGSYNR 250
LGESEHKRTSNVLFICATTENPSSALLKTFLRRIPMTIHIPSLEERSLNE 300
RVDLTTFLLGKEAERIKKNLSVHIDVYNALIHSAKFGNVGQLKSNVQLVC 350
AHGFLHNLDRNEVIELTVRDLPDEIKQEWMSSSKNMQRSKAISEYVNITT 400
IISPIVEDETTKIDEDLSFNLYHLIEEKVKTLMKEGLSKKDINQYILTDV 450
HLHVRSFFHHQAFQKDNLLTFVEDDVIQMTKQLKEIAEHELDCTFDRKFI 500
YFLSMHIDAFLKRGKQIDVLNTQETDEIRDTHVKEYRVAMIFKDKIQEYF 550
KVAIPEIEVIYLTMLIHSIKSLKENKRVGIIVAAHGNSTASSMVEVATEL 600
LGSTPIAAVDMPLTVSPSDILECVAEKMKQVDEGEGVLMLVDMGSLAMLE 650
SRLEEKTGISIKTISNVTTSMVLDAVRKVNYLNLNLHAIYQSVTKDFIEL 700
WERQPAASGKKKALVSICTTGSGTAKKLEDILTTIVNKASDTPIHILTVS 750
SIKLANSIKEIEKEYEILATVGTKDPKINAPHVSLEVLIEGEGEKLIQQA 800
ITKGSISLSNGLNEANIIVRELCEDSLKKYLVFLNPHHVIDMLLEWLQTV 850
QDELGVIFNNAVLIKVIMHTAFAFERVIKQNPIAFLEEEEEINDQLKEMVY 900
VTERTLAPYEEKLGLRISDDEKLFIAAIFAEEVHGQLF              938
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FIG. 2. Amino acid sequence deduced from DNA sequence of *levR*.

Comparison of the Amino Acid Sequence of the LevR Polypeptide with That of Known Regulatory Proteins. A computer search for similarities with other proteins revealed that the LevR polypeptide shares homologies with members of a class of regulatory proteins such as NifA and NtrC. A domain, which we called domain A (Fig. 3*A*), composed of 200 residues shares extensive similarity with NifA and NtrC of *K. pneumoniae* and NifA of *R. meliloti* (26, 27). Potential ATP-binding sites were observed in strongly conserved regions of the central region of NifA, NtrC, and in other homologous activators such as DctD from *Rhizobium leguminosarum* and XylR from *Pseudomonas putida* (7). These potential ATP-binding sites of domain A are present in several nucleotide-binding proteins and form part of the ATP-binding site of adenylate kinase (28). They are also present in LevR (boxed in Fig. 3*A*).

A second domain, domain B, of 161 residues is similar to that of members of another family of bacterial regulatory proteins known as transcriptional antiterminators: SacT and SacY from *B. subtilis* and BglG from *E. coli* (Fig. 3*B*). Conservative replacements were taken into account when comparing domain B to other proteins. The similarity between LevR and transcriptional antiterminators is surprising since no terminator was found downstream of the promoter of the operon (2). The four proteins share a common property: they are negatively controlled by the PTS (3, 8, 29, 30). This may explain the regions of sequence conservations.

The Promoter of the Levanase Operon. The fructose-inducible promoter of the levanase operon has previously been mapped in *B. subtilis* by primer extension (2). The −35 and −10 regions are weakly similar to those of σ^A-controlled promoters, as observed (2). Close inspection of DNA sequence revealed that the levanase promoter is similar to the −12, −24 promoters. The −12, −24 promoters control the expression of genes involved in nitrogen assimilation but also control unrelated metabolic functions (7, 31). The general features of this type of promoter are as follows: (*i*) They do not have the typical consensus sequences of *E. coli* promoters at −35 and −10 regions. They have a consensus 5′-CTGGCACN$_5$TTGCA-3′ sequence centered on positions

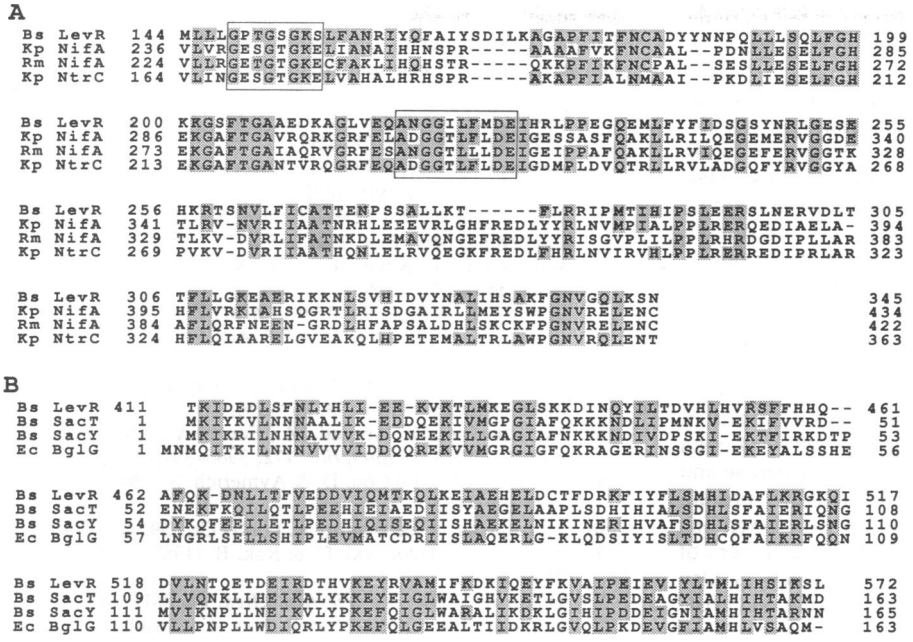

FIG. 3. Comparison of *B. subtilis* LevR with similar regulatory proteins. (*A*) Comparison of *B. subtilis* LevR with *K. pneumoniae* NifA, *R. meliloti* NifA, and *K. pneumoniae* NtrC. Amino acid sequence of the A domains of four polypeptides has been aligned by introducing gaps (hyphens) to maximize identity. Identical residues are boxed and numbers indicate the position of the residues in the respective protein. LevR is 33% identical in this domain with NifA of *K. pneumoniae* and *R. meliloti* and with NtrC of *K. pneumoniae*. (*B*) Comparison of *B. subtilis* LevR with *B. subtilis* SacT and SacY and *E. coli* BglG. Similar residues are boxed (accepted conservative replacements are I, L, V, and M; D and E; A and G; R and K; S and T; F and Y). The percentages of similarity between LevR and SacT, SacY, and BglG, in these domains, are 40%, 42%, and 34%, respectively.

−24, −12. (*ii*) They are recognized by a specific RNA polymerase σ^{54} factor encoded by *ntrA*. (*iii*) A positive regulatory protein interacts with upstream activating sequences (UASs) to stimulate the transcription (12, 31).

The DNA sequence of the levanase promoter revealed at −24 and −12 positions two sequences identical with those found in most NifA and σ^{54}-controlled promoters (Fig. 4). Eleven bases of the 12 are identical with the consensus when comparing the −12, −24 promoters. Moreover, a UAS-like element ($TGTN_{10}ACA$) centered at position −132 was also found upstream from the transcription start site of the levanase operon in the putative transcriptional terminator of the *levR* gene. This conservation of DNA sequences suggests that the levanase operon is controlled by a σ^{54}-type promoter in *B. subtilis*. Since the equivalent *ntrA* gene of *B. subtilis* has not yet been described, no σ^{54} defective mutant is available. Thus, we used a set of isogenic *E. coli* strains ET8000 (*ntrA*$^+$) and ET8045 (*ntrA*) to test the dependence of the promoter on σ^{54}. A series of plasmids was constructed containing translational *levD–lacZ* fusions in which the β-galactosidase is expressed from the levanase promoter. These plasmids contain the *levR*$^+$ gene (pRL6) or the *sacL8* allele (pRL7). A deleted plasmid without the *levR* gene was also constructed and used as a control (pRL5). The ET8000 and ET8045 strains were transformed with these plasmids (Table 2). β-Galactosidase was expressed constitutively in the ET8000 (*ntrA*$^+$) strain when the *levR*$^+$ or the *sacL8* allele was present on the plasmid. In this case, a 350-fold stimulation of expression was observed compared with the construction without *levR*. This stimulation was not observed when these plasmids were introduced into the ET8045 (*ntrA*) *E. coli* strain. This result indicates that σ^{54} is involved in the expression of the levanase operon at least in *E. coli*.

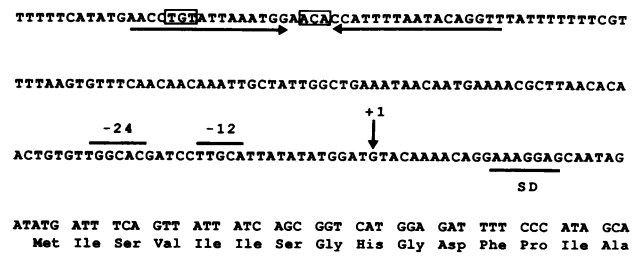

FIG. 4. Promoter region of the levanase operon. The sequence of a 227-bp fragment is presented, including the beginning of the *levD* coding sequence. The potential ribosome binding site (SD) is underlined. The transcription start point (+1) mapped in *B. subtilis* is indicated by a vertical arrow. The −12 and −24 regions corresponding to the transcription start point are overlined. Convergent arrows indicate the putative transcription terminator of the *levR* gene. Putative UAS for levR is boxed.

DISCUSSION

The results obtained in this work indicate that the upstream region of the levanase operon contains a positive regulatory *levR* gene involved in expression of the levanase operon. The five genes of this operon are transcribed from a single promoter, which has been previously characterized in *B. subtilis* (2). No transcriptional terminator with dyad symmetry was found between the promoter and the end of the operon. Two domains, A and B containing, respectively, 200 and 161 residues, were identified in LevR. The homology found between domain B and SacT, SacY, and BglG is surprising because the levanase operon promoter is probably not controlled by an antitermination mechanism. However, this may reflect the fact that these four proteins are all negatively controlled by the PTS. It has been shown that BglG, the antiterminator of the β-glucoside utilization system of *E. coli*, is regulated by phosphorylation via the PTS (30). The products of *levD* and *levE*, the first two genes of the levanase operon, are enzyme III-like, which also negatively

Table 2. Dependence of expression of the levanase operon in *E. coli* on *ntrA* and *levR* gene products

Plasmid	β-Galactosidase specific activity	
	Strain ET8000	Strain ET8045
pRL5 (Pf-*levD–lacZ*)	30	60
pRL6 (*levR*$^+$, Pf-*levD–lacZ*)	11,600	110
pRL7 [*levR*(*sacL8*), Pf-*levD–lacZ*]	11,400	90

E. coli ET8000 (*ntrA*$^+$) and ET8045 (*ntrA*) were transformed with pRL5, pRL6, and pRL7 plasmids. Cultures were grown at 37°C in M9 medium containing 0.4% glycerol, 0.1% L-glutamine, and ampicillin (100 μg/ml). β-Galactosidase specific activities were determined twice and are expressed as Miller units per mg of protein.

regulate operon expression (3). The phosphorylated enzyme III may transfer the phosphate group either to fructose or to domain B of LevR, inactivating its function.

The constitutive *sacL8* mutation, which eliminates 140 residues from the original polypeptide, was identified in *levR*. We may suppose that the carboxyl-terminal domain of LevR is involved in negative control of the activation process. One possible hypothesis is that the carboxyl-terminal domain of LevR masks a functional activator domain. An alternative hypothesis is that in the *sacL8* truncated polypeptide a conformational change rendered the LevR polypeptide insensitive to negative regulation by the PTS. Other examples of active truncated activators have been described (32, 33).

Domain A of LevR shares similarity with the well-conserved central domain of the NifA/NtrC family of bacterial activators. It was proposed that this central domain of NifA/NtrC is specifically required for the formation of open complexes between σ^{54} holoenzyme of RNA polymerase and the promoters (34, 35). ATP and a specific activator protein are necessary to catalyze formation of the corresponding open promoters. Indeed, the central domain of members of this family contains an ATP-binding site, which is also present in LevR. In Gram-negative bacteria, NifA and NtrC interact with promoters recognized by σ^{54} holoenzyme RNA polymerase. The consensus sequence of these promoters called −12, −24 promoters is now well established and differs from that of the −10, −35 vegetative promoters. The promoter of the levanase operon is very similar to the consensus of the −12, −24 promoters. It is also well known that NifA and NtrC interact with specific UASs to stimulate transcription. Actually, a putative UAS is present far upstream (−132) from the promoter of the levanase operon. Deletion mapping experiments performed in *B. subtilis* suggest that this putative UAS is involved in transcription activation of the levanase operon (I.M.-V., unpublished results).

No gene encoding σ^{54} has yet been identified in *B. subtilis*. Using an *E. coli ntrA* mutant, we showed that levanase operon expression is strictly dependent on the presence of both LevR from *B. subtilis* and σ^{54} from *E. coli*. However, we cannot exclude the possibility that σ^{54} also controls the promoter of *levR* in *E. coli*. Nevertheless, it is likely that *B. subtilis* contains a σ^{54}-like factor. It has been suggested that these −12, −24 promoters control a large family of regulons in Gram-negative bacteria (7, 31). Diverse functions including nitrogen fixation, C_4 dicarboxylate transport, assimilation of poor nitrogen sources, catabolism of aromatic compounds such as toluene and xylene, pilin formation, and pathogenicity are controlled by σ^{54}. These functions are expressed in response to certain environmental conditions and therefore need to have sensory systems that transduce the appropriate signals to the cognate regulators. The levanase operon may fit this pattern. The physiological functions of levanase after carbon source depletion could be the degradation of levans, which are polymers of fructose. It has already been shown that very low concentrations of fructose induce levanase synthesis via a signal transduction by a fructose-PTS.

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