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The *sacT* Gene Regulating the *sacPA* Operon in *Bacillus subtilis* Shares Strong Homology with Transcriptional Antiterminators

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The expression of the *Bacillus subtilis sacPA* operon is induced by sucrose. A DNA fragment containing the upstream region of this operon was cloned. This fragment contains a promoter from which the operon is expressed. This upstream region also contains a palindromic DNA sequence very similar to the transcriptional terminator which regulates the induction of the *B. subtilis sacB* gene. Of 37 nucleotides in a region partially overlapping the *sacP* palindromic sequence, 34 were identical to the corresponding region of the *sacB* gene. A similar motif is also present in the *bgl* operon of *Escherichia coli*. The *sacT* locus controlling *sacPA* expression had been identified by a single constitutive mutation *sacT30* which mapped close to the *sacPA* operon. DNA fragments containing the *sacT*$^+$ and *sacT30* alleles were cloned and sequenced. The *sacT* gene product is very similar to the *B. subtilis sacY* and to the *E. coli bglG* gene products. The constitutive *sacT30* mutation was identified. It corresponds to a Asp-96-to-Tyr missense mutation located in a highly conserved region in SacT and SacY. These results strongly suggest that *sacT* is a specific regulatory gene of the *sacPA* operon.

The structural genes involved in the utilization of sucrose in *Bacillus subtilis* are clustered in two regions of the bacterial chromosome (18). *sacA* codes for an endocellular sucrase (9, 10, 17), and *sacP* is the structural gene of a membrane-associated, specific component of the phosphoenolpyruvate-dependent carbohydrate phosphotransferase system (PTS) of sucrose transport (7, 8). *sacP* and *sacA* appear to be organized in an operon. A second sucrose-hydrolyzing enzyme, levansucrase, is encoded by *sacB* (20, 36). Both the expression of the *sacPA* operon and that of the *sacB* gene are inducible by sucrose (18). The sucrose regulon of *B. subtilis* has been extensively studied by genetic analysis. Mutants causing constitutive expression of saccharolytic enzymes have been selected, and their analysis led to the identification of three regulatory loci, *sacT*, *sacS*, and *sacR*. A single *sacT*(Con) mutant has been isolated, and the mutation *sacT30* was mapped close to the *sacPA* operon by transformation. In the *sacT30* mutant, both *sacP* and *sacA* expression are constitutive (18). The *sacS* locus was identified by several different constitutive mutations. The *sacS*(Con) mutations lead to constitutive expression of the *sacP*, *sacA*, and *sacB* genes. It was therefore postulated that the product of the *sacS* locus regulates the expression of *sacPA* and *sacB* (18, 19). The *sacS* locus was cloned (4, 6) and sequenced (38; M. Zukowski, cited in reference 8). It contained two genes, *sacX* and *sacY*. The *sacX* gene product negatively regulates *sacY* (4). The product of the *sacY* gene is very similar to the antiterminator of the *Escherichia coli bgl* operon (38). Furthermore, it was shown that *sacB* expression is regulated by a transcriptional antitermination mechanism (31). This regulation involves the *sacY* gene product, but a direct interaction of the *sacY* gene product with the transcriptional apparatus or with the DNA has not been yet demonstrated. S1 nuclease mapping of the *sacB* promoter defined the transcription start site 199 base pairs (bp) upstream of the *sacB* coding sequence (31). Between the promoter and the *sacB* coding sequence, a region of dyad symmetry, of approximately 70 nucleotides, was shown to act as a transcriptional terminator (31). Deletions of this termination structure or single-base changes that destroy the dyad symmetry lead to constitutive synthesis of levansucrase. The *sacR*(Con) mutations have been shown to be changes of the nucleotide sequence in the putative stem-and-loop structure (31, 33).

Little was known about the specific regulation of the *sacPA* operon at the molecular level. A strain deleted for *sacX* and *sacY* was constructed. In this strain, the *sacPA* operon is still inducible by sucrose (35). This experiment strongly suggested that *B. subtilis* has two regulatory pathways allowing the induction of the *sacP*, *sacA*, and *sacB* genes.

In this work, we have cloned the region upstream of the *sacPA* operon and have identified and sequenced the *sacT* gene and the *sacT30* allele. We have shown that the SacT protein is required for *sacPA* expression. SacT is very similar to the *sacY* gene product and to the *E. coli* BglG antiterminator (30). These results show that *sacT* is a regulatory gene involved in the control of *sacPA* induction.

MATERIALS AND METHODS

Strains. *B. subtilis* strains used in this study are listed in Table 1. *E. coli* K-12 strain TG1 [Δ(*lac-proAB*) *supE thi hsD5*(F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ* ΔM15); T. J. Gibson, Ph.D. thesis, University of Cambridge, 1984] was used for plasmid constructions and as a host for M13 phages. *E. coli* was transformed as previously described (5) with selection on L broth plates containing ampicillin (100 μg/ml). Transformation of *B. subtilis* was as previously described (3, 14), and selection was carried out on SP (1, 18) or tryptose blood agar base plates (Difco Laboratories, Detroit, Mich.) containing chloramphenicol (5 μg/ml), kanamycin (5 μg/ml), or erythromycin (25 μg/ml).

Media. *E. coli* was grown in Lb broth, and *B. subtilis* was grown in Penassay antibiotic medium 3 (Difco) or C medium (70 mM $K_2HPO_4 \cdot 3H_2O$, 30 mM KH_2PO_4, 25 mM $(NH_4)_2SO_4$, 0.5 mM $MgSO_4$, 0.01 mM $MnSO_4$, 22 mg of

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TABLE 1. Genotypes and sources of strains used in this study[a]

Strain	Genotype	Source or reference
168	*trpC2*	Laboratory stock
QB39	*trpC2 sacT30*	18
1A510	*recE4 leuA8 arg-15 thrA5 stp*	26
PG554	*trpC2 metC3 ptsI6*	11
GM329	*trpC2 metC3 ΔptsX ΔptsH ΔptsI ermC*	12
QB6001	*trpC2 sacT::aphA3*	pTP3→168
QB6003	*trpC2 aphA3*	pTP5→168
QB6005	*recE4 leuA8 arg-15 thrA5 stp*	pBSG8-24→1A510
QB6006	*trpC2*	pBSG8-24→168
QB6007	*trpC2 sacT30*	pBSG8-24→QB39
QB6008	*recE4 leuA8 arg-15 thrA5 stp*	pBSG8-29→1A510
QB6009	*trpC2 sacT30*	pBSG8-29→QB39
QB6010	*trpC2 amyE*::(del3 *sacP-lacZ cat*)	pTP7→168
QB6011	*trpC2 sacT30 amyE*::(del3 *sacP-lacZ cat*)	pTP7→QB39
QB6012	*trpC2 amyE*::(del4 *sacP-lacZ cat*)	pTP9→168
QB6013	*trpC2 metC3 ptsI6 amyE*::(del3 *sacP-lacZ cat*)	pTP7→PG554
QB6014	*trpC2 ΔptsX ΔptsH ΔptsI ermC amyE*::(del3 *sacP-lacZ cat*)	GM329→QB6010
QB6015	*recE4 leuA8 arg-15 thrA5 stp*	pHT3101→1A510
QB6016	*recE4 leuA8 arg-15 thrA5 stp*	pTP1→1A510
QB6017	*recE4 leuA8 arg-15 thrA5 stp*	pTP2→1A510

[a] Arrows (→) indicate construction by transformation. *del* indicates a deletion of the upstream region of *sacP*. *aphA3* indicates the kanamycin resistance gene from *Streptococcus faecalis* (37). *cat* indicates the chloramphenicol acetyltransferase gene from pC194 (13).

ferric ammonium citrate per liter) supplemented with the following nutrients: 20 mM potassium succinate, 50 mM potassium glutamate, 0.05% casein hydrolysate (CSKCH medium), and 100 mg of auxotrophic requirements per liter.

Plasmids. Plasmid pBSG8-15 was constructed as follows. A *Nae*I-*Taq*I restriction fragment containing the *cat* gene from plasmid pC194 (13) was cloned between the *Sma*I and *Sal*I restriction sites of plasmid pUC8. The resulting plasmid was pOB106 (a gift from P. Stragier). The *Eco*RI-*Hin*dIII fragment of pOB106 containing the *cat* gene was cloned between the two *Eco*RV restriction sites of the *sacP* gene in plasmid pBSG8-12 (7) (a pUC9 derivative). The resulting plasmid was called pBS G8-15.

Plasmid pKa was constructed by cloning a 3.5-kilobase-pair (kbp) *Eco*RI restriction fragment containing the origin of replication of pHV1436 (13a) in pAT21, a derivative of pBR322 containing the *aphA3* (Km^r) gene encoding the 3′ 5″-aminoglycoside phosphotransferase type III of *Streptococcus faecalis* (37).

pMC11 was constructed as follows. A 4.5-kbp *Sma*I-*Kpn*I fragment containing the *lacZ* and *erm* genes from pTV32 (27) was purified and made blunt-ended by T4 DNA polymerase. It was cloned between the *Sma*I and *Sal*I restriction sites of pEB111 made blunt-ended by using Klenow fragment of DNA polymerase I (16).

Plasmid pBSG8-24 was obtained by cloning (i) a 1-kbp *Hin*dIII-*Stu*I fragment purified from pTP1 (Fig. 1) and (ii) a 4.5-kbp *Sma*I-*Hin*dIII fragment extracted from pMC11 and containing the entire *lacZ* and *erm* genes into the single *Hin*dIII site of plasmid pKa. This plasmid contains a transcriptional fusion. pBSG8-29, a derivative of pBSG8-24, was constructed as follows. A 4.5-kbp *Xmn*I restriction fragment containing the *sacPA* promoter, the entire *lacZ* gene, and part of the *erm* gene was purified from pBSG8-24. It was cloned into the *Hin*dIII site of plasmid pKa made blunt by using Klenow fragment.

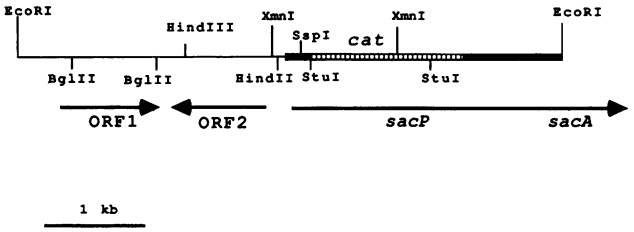

FIG. 1. Restriction map of the 4.5-kbp *Eco*RI DNA fragment containing the upstream DNA region of the *sacPA* operon in pTP1. The orientation of the *sacPA* operon is indicated with an arrow. The pHT3101 vector is not shown. The solid bar indicates the *sacPA* operon. The hatched bar indicates the *cat* gene. kb, Kilobase.

pTP3 was constructed as follows. A 1.5-kbp *Cla*I restriction fragment containing the *aphA3* gene was purified from plasmid pKa. This DNA fragment was cloned between the two *Bgl*II restriction sites of plasmid pTP1 replacing the 0.7-kbp *Bgl*II fragment. *Bgl*II and *Cla*I sites were made blunt by using the Klenow fragment of DNA polymerase I.

Plasmid pTP4 was constructed as follows. The 3-kbp *Eco*RI-*Bgl*II fragment containing the *cat* gene was purified from pTP1 (Fig. 1) and cloned into pBR322 between the *Eco*RI and *Bam*HI restriction sites. The resulting recombinant was called pTP4.

pTP5 was constructed by inserting the 1.5-kbp *Cla*I fragment containing the *aphA3* gene (see pTP3 construction above) into the single *Hin*dIII restriction site of pTP4.

pTP7 and pTP9 were constructed as follows. A *Xmn*I-*Ssp*I restriction fragment carrying the upstream region of the *sacPA* operon was cloned in the *Sma*I site of the *lacZ* gene of plasmid pIS112 (22) to create a hybrid gene. The translational fusion was then transferred to plasmid pAF1, which is a derivative of ptrpBG1 (32). pAF1 plasmid carries a promoterless *lacZ* gene between two fragments of the *B. subtilis amyE* gene. This facilitates single-copy integration of gene fusions at the *amyE* locus. pAF1 also contains the *cat* gene, allowing selection of integrants (10a). Unique *Stu*I and *Sac*I restriction sites are located in the *cat* gene and in the *lacZ* gene, respectively, of pIS112 plasmid. A *Stu*I-*Sac*I DNA fragment containing the translational fusion was inserted between the *Stu*I and *Sac*I restriction sites of pAF1 plasmid, leading to pTP7. Similarly, a *Hin*dII-*Ssp*I restriction fragment was cloned into the *Sma*I site of pIS112 and transferred in the same way to pAF1, giving plasmid pTP9. pTP7 and pTP9 plasmid constructions were verified by DNA sequencing with oligonucleotides located near the *Xmn*I and *Hin*dII restriction sites.

DNA manipulation. Standard procedures were used for extracting plasmids from *E. coli* and *B. subtilis* (1, 24). Restriction enzymes, T4 DNA polymerase, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were used according to the recommendations of the manufacturer. When necessary, 5′ and 3′ protruding ends of DNA fragments were repaired to blunt ends by using Klenow DNA polymerase, T4 DNA polymerase, and deoxynucleoside triphosphates. DNA fragments were purified from agarose gels by using the Gene Clean kit (Bio 101, Inc., La Jolla, Calif.).

DNA sequences were determined by the dideoxy-chain termination method with single-stranded M13 phages as templates (28) and modified T7 DNA polymerase (U.S. Biochemicals Corp., Cleveland, Ohio). The nucleotide sequence of the second DNA strand was determined by using synthetic oligonucleotides. Chromosomal DNA was purified

from exponentially growing cells as previously described (25).

β-Galactosidase assays. *B. subtilis* cells containing *lacZ* fusions were grown in the appropriate media. β-Galactosidase assays were carried out as described previously (25). *B. subtilis* colonies expressing *lacZ* fusions were detected by overlaying colonies with 8 ml of soft agar (7.5 mg/ml) containing lysozyme (2 mg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal; 250 μg/ml).

Cloning of the *sacT30* allele. The *sacT30* mutation was transferred from the chromosome of strain QB39 to the plasmid pTP1 as follows. Strain QB39 was transformed with pTP1. A single clone of this strain was grown in PAB medium containing erythromycin (25 μg/ml) and chloramphenicol (5 μg/ml) to an optical density at 600 nm of 1. Plasmid DNA was extracted and used to transform *B. subtilis* 1A510 (Rec$^-$). Individual colonies of 1A510 transformants were tested for sucrase synthesis. Thirty percent constitutively expressed sucrase. Plasmid DNA was extracted from one of these constitutive clones, introduced into *E. coli*, purified, and called pTP2. The restriction maps of pTP2 and pTP1 were identical (data not shown).

RESULTS

Cloning of the upstream region of the *sacPA* operon. The *sacT*(Con) mutation was previously mapped just upstream from the *sacPA* genes by transformation (18). The close linkage was used to clone the *sacT* locus. A DNA fragment containing the *cat* gene from plasmid pC194 was inserted into the *sacP* gene in pBSG8-12 (see Materials and Methods). The resulting recombinant plasmid pBSG8-15 was linearized and introduced into *B. subtilis* 168 by transformation. Spontaneous Cm^r transformants were selected which contained the *cat* gene integrated by double crossover in the *sacP* gene. The resulting strain, QB4503, was used to clone the upstream region of the *sacPA* operon into the stable shuttle vector pHT3101 in *B. subtilis* (21). For this purpose, *Eco*RI restriction fragments of QB4503 DNA were cloned into pHT3101 and the resulting plasmids were used to transform *B. subtilis* 168 or 1A510 (Rec$^-$) to Cm^r. Plasmid pTP1 (Cm^r Em^r) containing the upstream region of the *sacPA* operon on a 4.5-kbp *Eco*RI fragment was obtained in this way. A restriction map of this fragment is shown in Fig. 1.

DNA sequence of the *sacT* upstream region of the *sacPA* operon. The DNA sequence of a 2.0-kbp DNA fragment of the region upstream of the *sacPA* operon (Fig. 2) was determined on both strands by the dideoxy-chain termination method (see Materials and Methods). The sequenced region extends from 200 bp upstream of the *Bgl*II site (Fig. 1) to the beginning of the *sacP* coding sequence (Fig. 2). An open reading frame, open reading frame 1 (ORF1) (277 codons), was found starting at position 68 at a TTG putative initiation codon and ending at position 894. The TTG codon is preceded by a putative ribosome binding site (GGAG) with a low ΔG value (−39.3 kJ/mol). ORF1 encodes a polypeptide with a deduced molecular weight of 32,037 and was identified as the *sacT* gene (see below). The beginning of the *sacP* gene (7) was found at position 2008. A long palindromic structure containing two 31-bp stretches with nine mismatches is located at position 1940, centered 65 bp upstream of the translation initiation codon of *sacP* (Fig. 2). A second ORF, open reading frame 2 (ORF2), extends from right to left from position 1706 to position 936 (data not shown). A potential *rho*-independent terminator is centered at position 920 between the stop codon of ORF2 and the stop codon of *sacT* gene (Fig. 2).

Localization of the *sacPA* promoter. In order to define DNA sequences that play a role in the expression of the *sacPA* operon, a series of deletions was created upstream from the beginning of the *sacP* gene. Two transcriptional fusions with the *lacZ* of *E. coli* were constructed as shown in Fig. 3 and were cloned into the replicative plasmid pKa (see Materials and Methods). These two recombinant plasmids, pBSG8-24 and pBSG8-29, were introduced into *B. subtilis* 1A510 (Rec$^-$), giving strains QB6005 and QB6008, respectively. The uninduced levels of β-galactosidase expression observed in strains QB6005 and QB6008 are similar (Table 2). In the presence of sucrose, a 30- to 40-fold increase of β-galactosidase activity was observed in these strains. The values obtained are quite similar to that observed with the wild-type strain (Rec$^+$) containing the plasmid pBSG8-24 (QB6006). These results indicate that the *Xmn*I-*Stu*I restriction fragment cloned in front of the *lacZ* gene contains the *sacPA* sucrose-inducible promoter. This promoter is therefore located in the 245-bp fragment preceding the ATG initiation codon of *sacP* (Fig. 2).

In order to define further the location of the *sacPA* promoter, translational fusions of the amino-terminal region of *sacP* to the eighth codon of the *lacZ* gene were constructed and integrated as single copies at the *amyE* locus (see Materials and Methods; Fig. 3). Plasmids pTP7 and pTP9 (Fig. 3) were introduced into *B. subtilis* 168, giving strains QB6010 and QB6012, respectively. These strains were grown with and without induction with 0.1% sucrose. The β-galactosidase activity in strain QB6012 is sixfold lower than that observed in QB6010 in the presence of sucrose, suggesting that sequences located between *Xmn*I and *Hin*dII restriction sites are required for full *sacPA* expression.

Strain QB39 (*sacT30*) transformed either with pBSG8-24 (QB6007) or with pTP7 (QB6011) constitutively expresses β-galactosidase (Table 2). The absence of inducible recombinant clones indicates that the *sacT30* mutation is presumably located upstream of the *Hin*dIII site. As the promoter of the *sacP* gene, followed by the *lacZ* reporter gene, is located either on a replicative plasmid or at the chromosomal *amyE* locus, the *sacT* gene appears to be *trans* acting. This was confirmed as detailed below.

The constitutive expression of β-galactosidase in strains QB6007 and QB6011 is decreased eight- and sixfold, respectively, in the presence of sucrose. It was already shown that the constitutive synthesis of sucrase in the *sacT30* mutant is markedly repressed by carbon sources such as glucose, glycerol, or sucrose. This decrease is a consequence of catabolite repression, which has been previously observed for this and other enzymatic systems in *B. subtilis* (15, 19).

A comparison of DNA sequences in the promoter region of the *sacPA* and *sacB* genes is shown in Fig. 4. An imperfect palindromic sequence is present upstream of the *sacP* gene. Inspection of this sequence revealed a significant similarity to the terminator present in the *sacB* leader region (31). Fifty out of 53 bp are identical (Fig. 4, boxed regions). The largest box is homologous to box A and box B sequences found in the β-glucoside utilization system of *E. coli* and in the β-glucanase promoter region of *B. subtilis* (23).

Fine structure genetic analysis of the *sacT* locus. Gene disruption experiments were carried out in order to locate the *sacT* gene. ORF1 was disrupted by replacing the 0.7-kbp *Bgl*II fragment in plasmid pTP1 with a 1.5-kbp *Cla*I fragment containing the *aphA3* kanamycin resistance determinant

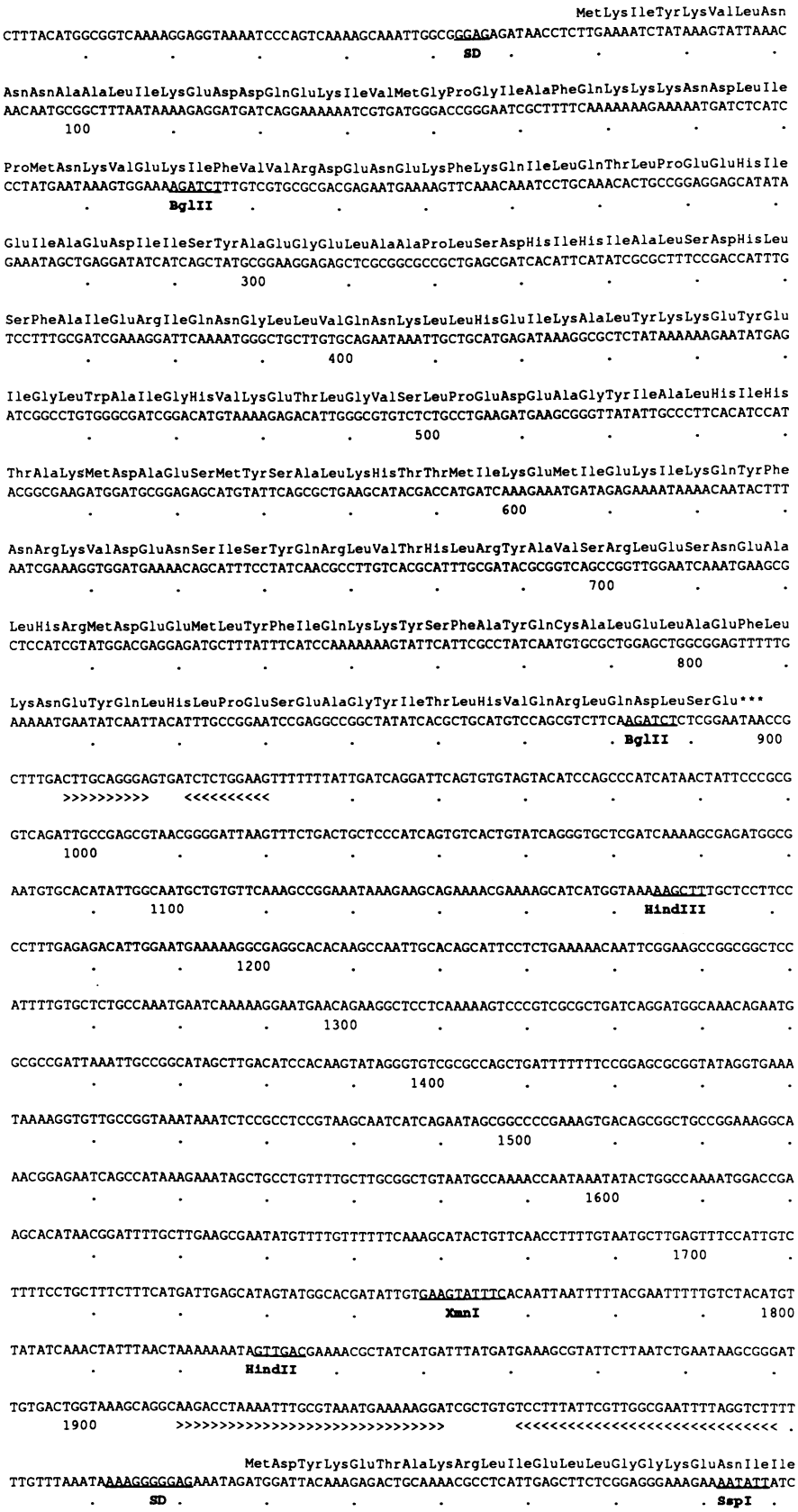

FIG. 2. DNA sequence of the *sacPA* upstream region (GenBank accession no. M33761). The deduced amino acid sequence of the *sacT* gene is shown. Potential Shine-Dalgarno sequences are underscored. The beginning of the *sacP* coding sequence is also shown (7). >>>><<<< indicates regions of dyad symmetry.

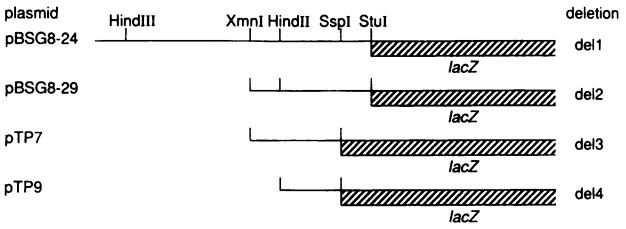

FIG. 3. Restriction maps of replicative plasmids (pBS G8-24 and pBS G8-29) and integrative plasmids (pTP7 and pTP9) containing different deletions of the upstream region of the *sacPA* operon.

yielding plasmid pTP3. In order to disrupt ORF2, *aphA3* was introduced at the unique *Hin*dIII site of pTP4 (see Materials and Methods), giving pTP5. These constructions were introduced into the chromosome of *B. subtilis* 168 by homologous recombination after transformation with linearized plasmids pTP3 and pTP5, giving strains QB6001 (ORF1::*aphA3*) and QB6003 (ORF2::*aphA3*), respectively (Fig. 5). Sucrase activities of strains QB6001 and QB6003 grown in CSKCH medium with and without induction with 0.1% sucrose (Fig. 5) were assayed as previously described (15) and compared with that of the wild-type strain. QB6001 (ORF1 disrupted) produced only very small amounts of sucrase, whereas QB6003 (ORF2 disrupted) showed normal sucrase activity. It was therefore concluded that while ORF1 is essential for the expression of the *sacPA* operon, ORF2 is not.

In order to confirm that ORF1 corresponds to *sacT*, the *sacT30* allele was cloned onto plasmid pTP2 in vivo by gene conversion as described in Materials and Methods. DNA sequencing of ORF1 and ORF2 upstream from *sacPA* revealed a single nucleotide change (G→T) at position 352 (Fig. 2) corresponding to an Asp-96-to-Tyr missense mutation in ORF1 (Fig. 6). This indicates that ORF1 is the *sacT* gene.

Dominance of *sacT*(Con) over *sacT*$^+$. Plasmids pTP1 (*sacT*$^+$), pTP2 (*sacT30*), and pHT3101 were introduced in *B. subtilis* 1A510 (Rec$^-$). Sucrase specific activity was assayed in CSKCH medium with and without induction with 0.1% sucrose. Constitutive sucrase expression was observed in the strain containing pTP2 (Table 3).

TABLE 2. Effect of upstream deletions on the expression of *sacP-lacZ* fusions

Strain	Relevant genotype	Replicative plasmid or chromosomal insertion[a]	β-Galactosidase activity[b]	
			−I	+I
QB6005	*recE4*	pBSG8-24(del1)	26	1,078
QB6006	Wild type	pBSG8-24(del1)	19	1,275
QB6007	*sacT30*	pBSG8-24(del1)	10,975	1,342
QB6008	*recE4*	pBSG8-29(del2)	28	760
QB6010	Wild type	*amyE*::(del3, *sacP-lacZ cat*)	17	573
QB6011	*sacT30*	*amyE*::(del3, *sacP-lacZ cat*)	4,690	733
QB6012	Wild type	*amyE*::(del4, *sacP-lacZ cat*)	13	95
QB6013	*ptsI6*	*amyE*::(del3, *sacP-lacZ cat*)	214	265
QB6014	Δ*ptsX* Δ*ptsH* Δ*ptsI*	*amyE*::(del3, *sacP-lacZ cat*)	684	725

[a] del, Deletions of the upstream region of *sacP* (see Fig. 3).
[b] Miller units per milligram of protein. −I, Uninduced; +I, induced.

This suggests that *sacT* may encode a *trans*-acting regulator of the *sacPA* operon. In the presence of sucrose, a strong decrease of sucrase activity was observed when the *sacT30* allele was present on the multiple-copy plasmid pTP2. A similar effect was observed in *B. subtilis* QB6007 and QB6011 (Table 2) and QB39 (*sacT30*) (15).

Similarity of SacT to other antiterminator proteins. The deduced amino acid sequence of *sacT* gene shares extensive similarity with the putative antiterminator SacY of the *B. subtilis* sucrose system and to a lesser extent with BglG, the antiterminator of the *E. coli* β-glucoside utilization system. The deduced SacT polypeptide is 48% identical to SacY and 35% identical to BglG (Fig. 6). The constitutive *sacT30* allele contains one missense mutation (Asp-96 to Tyr). A consti-

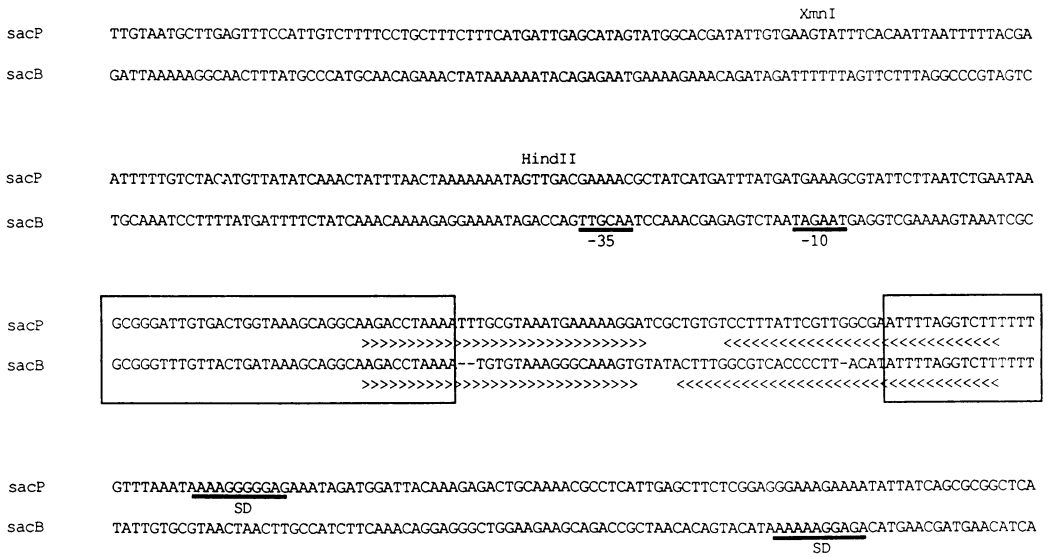

FIG. 4. Comparison of DNA sequences upstream of *sacP* and *sacB* genes. Identical nucleotides are boxed. Arrowheads indicate regions of dyad symmetry. Gaps were introduced to maximize the homologies. Putative Shine-Dalgarno sequences and −35 and −10 regions of the *sacB* promoter are underscored.

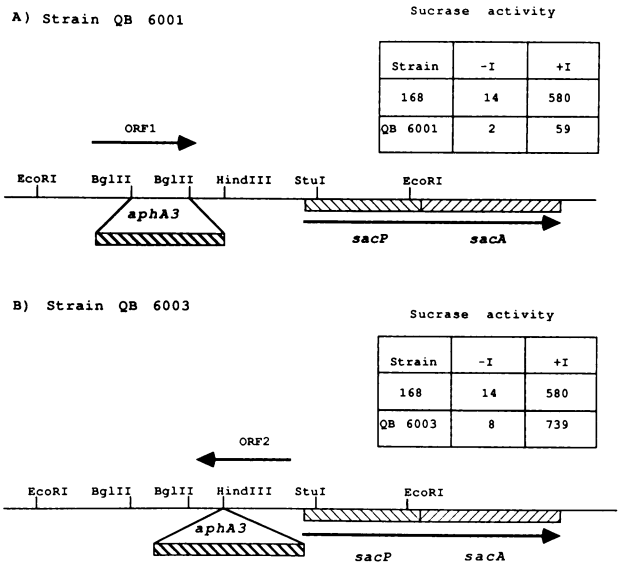

FIG. 5. Mapping of *sacT* by gene disruption. Strains QB6001 (A) and QB6003 (B) were grown in CSKCH medium with and without induction with 0.1% sucrose. Sucrase activity was compared with that of the wild-type strain grown in the same conditions and expressed in micromoles of glucose per minute per milligram of protein). −I, Uninduced; +I, induced.

tutive allele of the *sacY* gene (*sacS49*) (6) has previously been shown to contain a single missense mutation resulting in a His-to-Tyr change at amino acid 99 (M. Débarbouillé, unpublished results). Interestingly, both the *sacT30* and *sacS49* mutations are at sites in stretches of amino acid residues which are highly conserved in SacT, SacY, and BglG (Fig. 6).

Constitutive expression of the *sacPA* operon in a *ptsI* mutant. A previous report suggested that sucrase could be synthetized constitutively in a *B. subtilis* strain carrying a mutation of the enzyme I of the PTS (*ptsI6*) (M. Pascal, Ph.D. thesis, University of Paris VII, Paris, France, 1976). To confirm this report, the strain PG554, which carries the *ptsI6* mutation, was transformed with pTP7, which contains a translational fusion of *sacP* to *lacZ*. The resulting strain, QB6013, was grown with and without sucrose induction, and β-galactosidase was assayed. The β-galactosidase activity was identical in the induced and the uninduced cultures. This level was higher than that of the uninduced wild-type strain and 37% of that of the induced wild-type strain (Table 2). These results clearly indicate that *sacPA* operon expression is partially constitutive in a *ptsI* mutant in *B. subtilis*. A strain containing a deletion of *ptsX* (a *crr*-like gene), *ptsH* (coding for Hpr), and the 5′ end of *ptsI* (coding for the enzyme I) was constructed (12). This deletion was introduced by transformation into strain QB6010 to give strain QB6014. β-Galactosidase activities were determined after growth in CSK medium with and without 0.1% sucrose. The results presented in Table 2 indicate that expression of the *sacPA* operon is fully constitutive in strain QB6014. This result confirms that the PTS negatively controls the transcription of the *sacPA* operon.

DISCUSSION

Our results show that the *sacT* gene product is involved in *sacPA* operon expression. A 10-fold decrease of sucrase synthesis was observed in a Δ*sacT* strain, as compared with that observed in the wild-type strain. It was previously shown that two partially interchangeable regulatory pathways allowed the induction of two saccharolytic enzymes in *B. subtilis* (35). The finding that the *sacT* gene product is involved in *sacPA* expression supports this result. It has been shown that *sacB* transcripts stop at an upstream terminator in the absence of sucrose and extend past the terminator in the presence of sucrose (31). The *sacY* gene of the *sacS* locus encodes the putative antiterminator involved in this process. The *sacT* and *sacY* gene products share

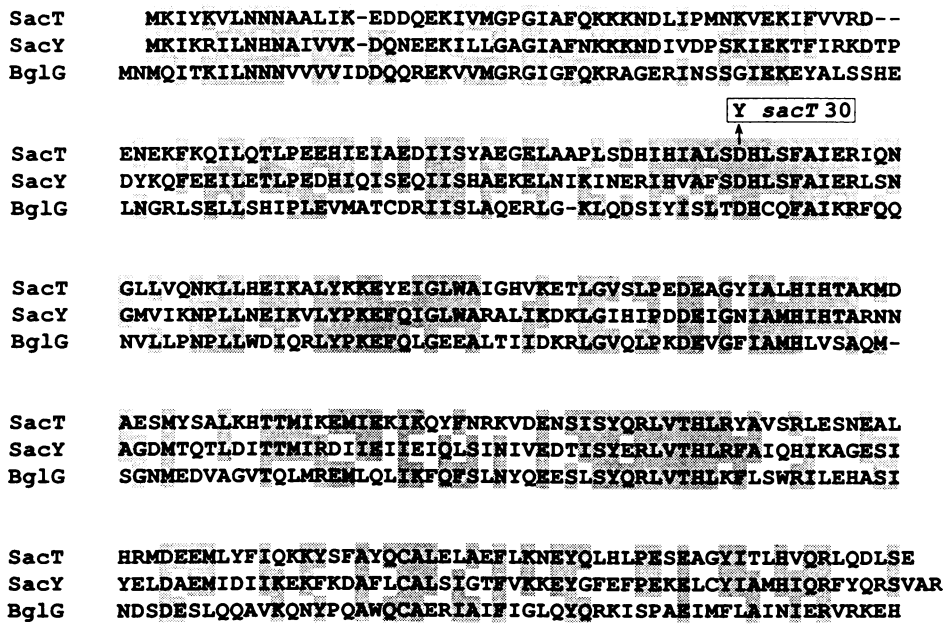

FIG. 6. Comparison of *sacT*, *sacY* of *B. subtilis*, and *bglG* gene products of *E. coli*. Gaps were introduced to optimize the homologies. Regions of identity are indicated by shaded boxes.

TABLE 3. Assays of sucrase activity in strains carrying *sacT*$^+$ or *sacT30* on a multiple-copy plasmid

Strain	Host and plasmids	Sucrase sp act (10^3)[a]	
		−I	+I
QB6015	1A510(pHT3101)	2	600
QB6016	1A510(pTP1)	8	430
QB6017	1A510(pTP2)	3,000	452

[a] Specific activity is defined as the amount of enzyme liberating 1 μmol of glucose per min per mg of protein. −I, uninduced; +I, induced.

strong similarity throughout their sequences, with 48% of the residues being identical. An intergenic region of 870 bp was found between the end of *sacT* and the *XmnI* site located in the promoter region of the *sacPA* operon. The *sacPA* promoter region was located within the 245 bp preceding the initiation codon of the *sacP* gene. This intergenic region contains an additional ORF whose disruption does not interfere with the expression of the *sacPA* operon. A palindromic DNA sequence is present 70 bp upstream from the initiation codon of *sacP*. This DNA sequence is similar to the *sacB* transcriptional terminator located between the promoter and the *sacB* coding sequence. Highly similar DNA sequences (34 of 37 bp) were found upstream of the palindromic structures of the *sacB* and the *sacPA* genes (Fig. 4). These sequences partially overlap the palindromic structures and correspond to box A and box B as previously described for the β-glucoside utilization operon of *E. coli* and for the β-glucanase gene of *B. subtilis* (data not shown) (30). It is tempting to speculate that *sacT* encodes an antiterminator interacting directly or indirectly with the palindromic structure upstream of *sacPA*.

The strong similarity of the two regulatory systems controlling *sacPA* and *sacB* genes raises the possibility of "cross-talk" between them. SacT is very similar to SacY, and their putative targets (palindromic sequences) are also very similar. Residual *sacB* induction was detected in a Δ*sacX* Δ*sacY* strain (35). This low level of *sacB* expression may be due at least in part to the *sacT* gene product. A low constitutive level of *sacB* expression is detected in *sacT30* mutants (Pascal, Ph.D. thesis). A reciprocal phenomenon was observed in a Δ*sacT* strain (QB6001); low-level expression of *sacPA* was induced by sucrose, suggesting the involvement of the *sacY* gene product. This hypothesis is reinforced by the fact that *sacB*, *sacP*, and *sacA* are constitutive in a *B. subtilis* strain containing a *sacY*(Con) allele, thus strongly suggesting that in a constitutive mutant, a modified SacY protein induces *sacPA* expression.

The involvement of a specific component of the PTS in induction has been shown for the *bgl* operon in *E. coli* (23). *bglF* is the structural gene encoding the enzyme II^{Bgl}, which is both a negative regulator of the operon expression and a component of the phosphotransferase involved in the uptake of β-glucosides. BglG is a transcriptional antiterminator. Phosphorylated BglF can transfer phosphate either to β-glucosides or to BglG, blocking its action as an antiterminator (2, 29). Another example of gene regulation in which a PTS is involved has recently been proposed for the levanase operon in *B. subtilis* (I. Martin-Verstraete, M. Débarbouillé, A. Klier, and G. Rapoport, J. Mol. Biol., in press). Preliminary evidence suggests that the product of the *sacX* gene of the *sacS* locus negatively controls *sacB* expression (4). The SacX protein shares a strong homology with the enzyme II^{Suc} of the sucrose PTS (38; M. Zukowski, cited in reference 8). The SacX protein could be a functional but minor enzyme II^{Suc} with a mainly regulatory role. It has been proposed that the SacX protein functions in a manner analogous to BglF, regulating SacY activity by phosphorylation (34). The *sacPA* operon is constitutively expressed at a low level in the *B. subtilis ptsI* mutant. However, in a strain deleted for *ptsX*, *ptsH*, and part of *ptsI*, the expression of the operon is fully constitutive, thus confirming the involvement of the PTS in the induction.

The three regulatory proteins, one encoded by the *bgl* operon of *E. coli* and two encoded by the sucrose regulon of *B. subtilis*, are homologous proteins which presumably function by similar antitermination mechanisms. Interestingly, constitutive mutations in the three antiterminators have been found in the same region (Fig. 6). The *sacT30* mutation corresponds to an Asp-96-to-Tyr modification, and *bglG33* corresponds to an Asp-99-to-Asn change (S. Rankin and A. Wright, personal communication). It is therefore tempting to speculate that this region, which includes conserved Asp and His residues, is involved in the regulation of the activity of the antiterminator, probably by phosphorylation. The expression of the *sacPA* operon remains inducible in a Δ*sacX* Δ*sacY* strain of *B. subtilis*. The *sacX* gene product is therefore not required for the control of the SacT antiterminator. By analogy with the *sacX sacY* system, the *sacT* gene product is expected to be regulated by SacP or by another enzyme II-like regulator. Biochemical evidence is needed to analyze and confirm the role of SacT as an antiterminator. In addition, the regulation of the activity of SacT remains to be determined.

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LITERATURE CITED

1. **Amory, A., F. Kunst, E. Aubert, A. Klier, and G. Rapoport.** 1987. Characterization of the *sacQ* genes from *Bacillus licheniformis* and *Bacillus subtilis*. J. Bacteriol. **169**:324–333.
2. **Amster-Choder, O., F. Houman, and A. Wright.** 1989. Protein phosphorylation regulates transcription of the β-glucoside utilization operon in *E. coli*. Cell **58**:847–855.
3. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81**:741–746.
4. **Aymerich, S., and M. Steinmetz.** 1987. Cloning and preliminary characterization of the *sacS* locus from *Bacillus subtilis* which controls the regulation of the exoenzyme levansucrase. Mol. Gen. Genet. **208**:114–120.
5. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Non chromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. USA **69**:2110–2114.
6. **Débarbouillé, M., F. Kunst, A. Klier, and G. Rapoport.** 1987. Cloning of the *sacS* gene encoding a positive regulator of the sucrose regulon in *Bacillus subtilis*. FEMS Microbiol. Lett. **41**:137–140.
7. **Fouet, A., M. Arnaud, A. Klier, and G. Rapoport.** 1987. *Bacillus subtilis* sucrose-specific enzyme II of the phosphotransferase system: expression in *Escherichia coli* and homology to enzymes II from enteric bacteria. Proc. Natl. Acad. Sci. USA **84**:8773–8777.
8. **Fouet, A., M. Arnaud, A. Klier, and G. Rapoport.** 1989. Genetics of the phosphotransferase system of *Bacillus subtilis*. FEMS Microbiol. Lett. **63**:175–182.

9. **Fouet, A., A. Klier, and G. Rapoport.** 1982. Cloning and expression in *Escherichia coli* of the sucrase gene from *Bacillus subtilis*. Mol. Gen. Genet. **186**:399–404.
10. **Fouet, A., A. Klier, and G. Rapoport.** 1986. Nucleotide sequence of the sucrase gene of *Bacillus subtilis*. Gene **45**: 221–225.

10a. **Fouet, A., and A. L. Sonenshein.** 1990. A target for carbon source-dependent negative regulation of the *citB* promoter of *Bacillus subtilis*. J. Bacteriol. **172**:835–844.

11. **Gay, P., P. Cordier, M. Marquet, and A. Delobbe.** 1973. Carbohydrate metabolism and transport in *Bacillus subtilis*. A study of *ctr* mutations. Mol. Gen. Genet. **121**:355–368.
12. **Gonzy-Tréboul, G., M. Zagorec, M.-C. Rain-Guion, and M. Steinmetz.** 1989. Phosphoenol pyruvate:sugar phosphotransferase system of *Bacillus subtilis*: nucleotide sequence of *ptsX*, *ptsH* and the 5′-end of *ptsI* and evidence for a *ptsHI* operon. Mol. Microbiol. **3**:103–112.

13a. **Janniere, L., C. Bruand, and S. D. Ehrlich.** 1990. Structurally stable *Bacillus subtilis* cloning vectors. Gene **87**:53–61.

13. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pC194, a plasmid that specifies inducible chloramphenicol resistance. J. Bacteriol. **150**:815–825.
14. **Kunst, F., M. Débarbouillé, T. Msadek, M. Young, C. Mauel, D. Karamata, A. Klier, G. Rapoport, and R. Dedonder.** 1988. Deduced polypeptides encoded by the *Bacillus subtilis sacU* locus share homology with two-component sensor-regulator systems. J. Bacteriol. **170**:5093–5101.
15. **Kunst, F., M. Pascal, J.-A. Lepesant, J. Walle, and R. Dedonder.** 1974. Purification and some properties of an endocellular sucrase from a constitutive mutant of *Bacillus subtilis* Marburg 168. Eur. J. Biochem. **42**:611–620.
16. **Leonhardt, H., and J. C. Alonso.** 1988. Construction of a shuttle vector for inducible gene expression in *Escherichia coli* and *Bacillus subtilis*. J. Gen. Microbiol. **134**:605–609.
17. **Lepesant, J.-A., A. Billault, J. Kejzlarová-Lepesant, M. Pascal, F. Kunst, and R. Dedonder.** 1974. Identification of the structural gene for sucrase in *Bacillus subtilis* Marburg. Biochimie **56**: 1465–1470.
18. **Lepesant, J.-A., F. Kunst, J. Lepesant-Kejzlarová, and R. Dedonder.** 1972. Chromosomal location of mutations affecting sucrose metabolism in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **118**:135–160.
19. **Lepesant, J.-A., F. Kunst, M. Pascal, J. Kejzlarová-Lepesant, M. Steinmetz, and R. Dedonder.** 1976. Specific and pleiotropic regulatory mechanisms in the sucrose system of *Bacillus subtilis* 168, p. 58–69. *In* D. Schlessinger (ed.), Microbiology—1976. American Society for Microbiology, Washington, D.C.
20. **Lepesant, J.-A., J. Lepesant-Kejzlarová, M. Pascal, F. Kunst, A. Billault, and R. Dedonder.** 1974. Identification of the structural gene of levansucrase in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **128**:213–221.
21. **Lereclus, D., O. Arantes, J. Chaufaux, and M.-M. Lecadet.** 1989. Transformation and expression of a cloned δ-endotoxin gene in *Bacillus thuringiensis*. FEMS Microbiol. Lett. **60**:211–218.
22. **Lewandoski, M., and I. Smith.** 1988. Use of a versatile *lacZ* vector to analyse the upstream region of the *Bacillus subtilis spoOF* gene. Plasmid **20**:148–154.
23. **Mahadevan, S., A. E. Reynolds, and A. Wright.** 1987. Positive and negative regulation of the *bgl* operon in *Escherichia coli*. J. Bacteriol. **169**:2570–2578.
24. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Msadek, T., F. Kunst, D. Henner, A. Klier, G. Rapoport, and R. Dedonder.** 1990. Signal transduction pathway controlling synthesis of a class of degradative enzymes in *Bacillus subtilis*: expression of the regulatory genes and analysis of mutations in *degS* and *degU*. J. Bacteriol. **172**:824–834.
26. **Ostroff, G. R., and J. J. Pène.** 1983. Molecular cloning with bifunctional plasmid vectors in *Bacillus subtilis*: isolation of a spontaneous mutant of *Bacillus subtilis* with enhanced transformability for *Escherichia coli*-propagated chimeric plasmid DNA. J. Bacteriol. **156**:934–936.
27. **Perkins, J. B., and P. J. Youngman.** 1986. Construction and properties of Tn*917-lac*, a transposon derivative that mediates transcriptional gene fusions in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **83**:140–144.
28. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
29. **Schnetz, K., and B. Rak.** 1988. Regulation of the *bgl* operon of *Escherichia coli* by transcriptional antitermination. EMBO J. **7**:3271–3277.
30. **Schnetz, K., C. Toloczyki, and B. Rak.** 1987. β-Glucoside (*bgl*) operon of *Escherichia coli* K-12: nucleotide sequence, genetic organization, and possible evolutionary relationship to regulatory components of two *Bacillus subtilis* genes. J. Bacteriol. **169**:2579–2590.
31. **Shimotsu, H., and D. J. Henner.** 1986. Modulation of *Bacillus subtilis* levansucrase gene expression by sucrose and regulation of the steady-state mRNA level by *sacU* and *sacQ* genes. J. Bacteriol. **168**:380–388.
32. **Shimotsu, H., and D. J. Henner.** 1986. Construction of a single-copy integration vector and its use in analysis of regulation of the *trp* operon *Bacillus subtilis*. Gene **43**:85–94.
33. **Steinmetz, M., and S. Aymerich.** 1986. Analyse génétique de *sacR*, régulateur en *cis* de la synthèse de la lévane-saccharase de *Bacillus subtilis*. Ann. Inst. Pasteur Microbiol. **137A**:3–14.
34. **Steinmetz, M., S. Aymerich, G. Gonzy-Tréboul, and D. Le Coq.** 1988. Levansucrase induction by sucrose in *Bacillus subtilis* involves an antiterminator. Homology with the *Escherichia coli bgl* operon, p. 11–15. *In* A. T. Ganesan and J. A. Hoch (ed.), Genetics and biotechnology of *bacilli*, vol. 2. Academic Press, Inc., New York.
35. **Steinmetz, M., D. Le Coq, and S. Aymerich.** 1989. Induction of saccharolytic enzymes by sucrose in *Bacillus subtilis*: evidence for two partially interchangeable regulatory pathways. J. Bacteriol. **171**:1519–1523.
36. **Steinmetz, M., D. Le Coq, S. Aymerich, G. Gonzy-Tréboul, and P. Gay.** 1985. The DNA sequence of the gene for the secreted *Bacillus subtilis* enzyme levansucrase and its genetic control sites. Mol. Gen. Genet. **200**:220–228.
37. **Trieu-Cuot, P., and P. Courvalin.** 1983. Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3′ 5″-aminoglycoside phosphotransferase type III. Gene **23**:331–341.
38. **Zukowski, M., L. Miller, P. Cogswell, and K. Chen.** 1988. Inducible expression system based on sucrose metabolism genes of *Bacillus subtilis*, p. 17–22. *In* A. T. Ganesan and J. A. Hoch (ed.), Genetics and biotechnology of *bacilli*, vol. 2. Academic Press, Inc., New York.