JOURNAL OF BACTERIOLOGY, Oct. 1995, p. 5590–5597 Vol. 177, No. 19
0021-9193/95/$04.00+0

Regulation of the Putative *bglPH* Operon for Aryl-β-Glucoside Utilization in *Bacillus subtilis*

STEFFEN KRÜGER AND MICHAEL HECKER*

Institut für Mikrobiologie und Molekularbiologie, Ernst-Moritz-Arndt-Universität, D-17487 Greifswald, Germany

Received 8 June 1995/Accepted 3 August 1995

The expression of the putative operon *bglPH* of *Bacillus subtilis* was studied by using *bglP′-lacZ* transcriptional fusions. The *bglP* gene encodes an aryl-β-glucoside-specific enzyme II of the phosphoenolpyruvate sugar:phosphotransferase system, whereas the *bglH* gene product functions as a phospho-β-glucosidase. Expression of *bglPH* is regulated by at least two different mechanisms: (i) carbon catabolite repression and (ii) induction via an antitermination mechanism. Distinct deletions of the promoter region were created to determine *cis*-acting sites for regulation. An operatorlike structure partially overlapping the −35 box of the promoter of *bglP* appears to be the catabolite-responsive element of this operon. The motif is similar to that of *amyO* and shows no mismatches with respect to the consensus sequence established as the target of carbon catabolite repression in *B. subtilis*. Catabolite repression is abolished in both *ccpA* and *ptsH1* mutants. The target of the induction by the substrate, salicin or arbutin, is a transcriptional terminator located downstream from the promoter of *bglP*. This structure is very similar to that of transcriptional terminators which regulate the induction of the *B. subtilis sacB* gene, the *sacPA* operon, and the *Escherichia coli bgl* operon. The *licT* gene product, a member of the BglG-SacY family of antitermination proteins, is essential for the induction process. Expression of *bglP* is under the negative control of its own gene product. The general proteins of the phosphoenolpyruvate-dependent phosphotransferase system are required for *bglP* expression. Furthermore, the region upstream from *bglP*, which reveals a high AT content, exerts a negative regulatory effect on *bglP* expression.

The ability of bacteria to utilize aryl-β-glucosides can be considered as a strategy to exhaust alternative carbon sources under conditions of nutritional limitation. In *Escherichia coli*, there are several genes whose products are involved in the utilization of such compounds (18, 33, 35, 42), but most of them are cryptic in wild-type strains. They can be activated by insertion of insertion elements or by base substitutions near the catabolite gene activator protein-cyclic AMP (cAMP) binding site (38). The *bgl* operon in *E. coli* consists of *bglG*, *bglF*, and *bglB* (42). The phospho-β-glucosidase BglB hydrolyzes phosphorylated forms of arbutin and salicin. Gene *bglG* encodes a positive regulator that acts as an antitermination protein via binding to its mRNA target (RAT) in the presence of β-glucosides (2, 22). The RAT sequence partially overlaps a termination structure upstream of the *bglG* gene. The BglF protein, a component of the phosphoenolpyruvate-dependent phosphotransferase system (PTS), is responsible for the uptake and phosphorylation of the aromatic β-glucosides arbutin and salicin. It is a crucial element in the regulation of the *bgl* operon; i.e., BglF inactivates the antiterminator BglG by phosphorylation in the absence of substrate (1). When β-glucosides become available, the phosphorylated BglG is rapidly dephosphorylated and thus contributes to induction of the *bgl* operon (41).

In *Bacillus subtilis*, the genes *bglP* and *bglH* have recently been cloned and sequenced. The protein sequences show similarities to those of the enzymes II of the PTS and phospho-β-glucosidases, respectively (28). Complementation studies showed that BglP can act as a β-glucoside permease and that BglH can hydrolyze salicin in *E. coli* with the *bgl* genes deleted. It has been reported that the expression of *bglP* is inducible by salicin, and it has been suggested that the induction might be mediated via a termination-antitermination mechanism (28). Such a mechanism has been described for the sucrose utilization systems *sacPA* and *sacB* (4, 5, 9–11) of *B. subtilis*. Moreover, the activity of the antitermination protein LicT, which is involved in the regulation of β-glucan utilization in *B. subtilis* (46), appears to be altered by BglP.

Several systems for the utilization of β-glucosides are present in *B. subtilis*. A gene encoding a phospho-β-glucosidase (*bglA*) showing considerable sequence similarities to the *E. coli bglB* gene has been cloned and characterized. The expression of this gene is inducible by arbutin and salicin, but its inactivation has no phenotypic effect (51). Recently, another enzyme II inducible by salicin (*bglX*) has been cloned and sequenced. A disruption of this gene did not alter the ability to grow on salicin (16). Interestingly, all systems map around 330° of the *B. subtilis* genome.

In this study, the expression and regulation of the putative *bglPH* operon of *B. subtilis* were investigated. Distinct deletions of the regulatory region were constructed to determine *cis*-acting elements. Several mutations in potential *trans*-acting regulators were combined with such deletions. This approach led us to suggest a scheme for the complex regulation of the expression of *bglP*.

* Corresponding author. Mailing address: Institut für Mikrobiologie und Molekularbiologie, Ernst-Moritz-Arndt-Universität, Jahnstrasse 15, 17487 Greifswald, Germany. Phone: 03834-77271, ext. 210. Fax: 03834883353. Electronic mail address: glucose@microbio1.biologie.uni-greifswald.de.

MATERIALS AND METHODS

Bacterial strains and growth conditions. All *B. subtilis* strains used in this study are listed in Table 1. *E. coli* RR1 [F⁻ Δ(*gpt-proA*)*62 mcrB mrr ara-14 lacY1 leuB6 galK2 rpsL20 xyl-5 mtl-1 supE44*] (7) was used for the construction of

TABLE 1. *B. subtilis* strains used in this study

Strain	Genotype or relevant characteristics[a]	Source[b] or reference
IS58	*trpC2 lys-3*	27
QB6046	*sacT30 ptsG*::*cat amyE*::(*sacP′-lacZ aphA3*)	3
GM273	*trpC2 sacR-lacZ* Δ*ptsGHI*::*erm*	9
GM1038	*sacA321 sacB*Δ*23 ccpA*::Tn*917′*(Δ*erm*, *lacZ*)::*phl*	44
GM1221	*trpC2 pheA1* Δ*bgaX amyE*::(*gntRK′-lacZ phl*) *ptsH*$^+$ (*cat*)	13
GM1222	*trpC2 pheA1* Δ*bgaX amyE*::(*gntRK′-lacZ phl*) *ptsH1* (*cat*)	13
BGW10	*trpC2 lys-3* Δ*licTS*::*erm*	This work
BGW40	*trpC2 lys-3 amyE*::(*lacZ cat*)	This work
BGW41	*trpC2 lys-3 amyE*::(*bglP′-lacZ cat*)	28
BGW46	*trpC2 lys-3 amyE*::(*bglP*Δ*3′-lacZ cat*)	pSL3 tf→IS58
BGW47	*trpC2 lys-3 amyE*::(*bglP*Δ*2′-lacZ cat*)	pSL2 tf→IS58
BGW101	*trpC2 lys-3 amyE*::(*bglP′-lacZ cat*) Δ*licTS*::*erm*	pSL4[c] tf→BGW10
BGW104	*trpC2 lys-3 amyE*::(*bglP*Δ*14′-lacZ cat*) Δ*licTS*::*erm*	pSL14 tf→BGW10
BGW107	*trpC2 lys-3 amyE*::(*bglP*Δ*15′-lacZ cat*) Δ*licTS*::*erm*	pSL15 tf→BGW10
BGW401	*trpC2 lys-3 amyE*::(*bglP*Δ*12′-lacZ cat*)	pSL12 tf→IS58
BGW402	*trpC2 lys-3 amyE*::(*bglP*Δ*13′-lacZ cat*)	pSL13 tf→IS58
BGW403	*trpC2 lys-3 amyE*::(*bglP*Δ*10′-lacZ cat*)	pSL10 tf→IS58
BGW406	*trpC2 lys-3 amyE*::(*bglP*Δ*14′-lacZ cat*)	pSL14 tf→IS58
BGW407	*trpC2 lys-3 amyE*::(*bglP*Δ*15′-lacZ cat*)	pSL15 tf→IS58
BGW408	*trpC2 lys-3 amyE*::(*bglP*Δ*16′-lacZ cat*)	pSL16 tf→IS58
BGW409	*trpC2 lys-3 amyE*::(*bglP*Δ*17′-lacZ cat*)	pSL17 tf→IS58
BGW410	*trpC2 lys-3 amyE*::(*bglP′-lacZ cat*::*neo*)	pCm:Nm[d] tf→BGW41
BGW415	As BGW41 plus Δ*bglP*::*spe*	pIC260[c] tf→BGW41
BGW417	As BGW41 plus *ccpA*::Tn*917′*(Δ*erm*, *lacZ*)::*phl*	GM1038 tf→BGW41
BGW420	As BGW41 plus Δ*ptsGHI*::*erm*	GM273 tf→BGW41
BGW4101	As BGW410 plus *ptsH*$^+$ (*cat*)	GM1221 tf→BGW410
BGW4102	As BGW410 plus *ptsH1* (*cat*)	GM1222 tf→BGW410
BGW4103	As BGW410 plus *ptsG*::*cat*	QB6046 tf→BGW410
BGW4171	As BGW403 plus *ccpA*::Tn*917′* (Δ*erm*, *lacZ*)::*phl*	GM1038 tf→BGW403

[a] *erm*, erythromycin resistance; *cat*, chloramphenicol acetyltransferase gene; *aphA3*, kanamycin resistance; *neo*, neomycin resistance; *spe*, spectinomycin resistance; *phl*, phleomycin resistance.
[b] tf→, transformation.
[c] Described in reference 28.
[d] Described in reference 44.

recombinant plasmids and preparation of templates for DNA sequencing. This strain was grown in nutrient broth medium as described previously (27). For selection, ampicillin was added to a final concentration of 100 mg/liter. *B. subtilis* cells were grown in amino acid starvation medium (ASM) or in glucose limitation medium as described previously (47). ASM was supplemented with 0.1% glucose, which was omitted when another substrate served as a carbon source. If necessary, antibiotics were added to final concentrations of 5 (chloramphenicol), 1 (erythromycin), 25 (lincomycin), 10 (kanamycin), 10 (neomycin), 100 (spectinomycin), or 0.2 (phleomycin) mg/liter.

Oligonucleotides and PCR. The sequences of oligonucleotides used as primers for PCR experiments are as follows: P-1, 5′-d(GAAGTTAATACTAAGTTCAAATC)3′; P-2, 5′-d(TTGACATCTCACGAATCTAG)3′; P-2cre, 5′-d(TGAAAGCGTTGACATCTCACG)3′; P-3r, 5′-d(CATTTTTGGTGTTGACTATCG)3′; and P-4r, 5′-d(GCTTTCGCAGTAACAATCCTG)3′. The arrangement of the primers in the regulatory region of *bglP* is shown in Fig. 1. Flanking primers that hybridize upstream and downstream of the polylinker of pUC19 (50) were described previously (27). Generation of PCR products was done as described previously (27). Briefly, 50 pmol of each primer was used. To decrease the occurrence of potential mismatch bases, about 1 to 2 μg of plasmid DNA or 5 to 8 μg of chromosomal DNA was used and only 20 cycles were run. PCR products were cloned according to the Double GeneClean protocol (Bio101 Inc.) and were subsequently sequenced (described below) to detect *Taq* DNA polymerase artifacts.

Plasmids. The 1.1-kb *Bam*HI-*Pvu*II fragment of pIC334 (28) containing the regulatory part and the first few codons of *bglP* was cloned into pUC19, yielding pUC19/5. This plasmid was used to generate PCR products (described below). Plasmid pDH32M (26), which contains a promoterless *lacZ* gene and two fragments of the *B. subtilis amyE* gene, was used to construct transcriptional *lacZ* fusions. A *cat* gene allows the selection of integrants.

Plasmids pSL2 and pSL3 were obtained as follows. A 210-bp *Acc*I fragment and a 410-bp *Xba*I-*Pvu*II fragment, respectively, were isolated from pIC334. The ends were filled in with the Klenow fragment of DNA polymerase I and ligated into the single *Sna*BI site of pDH32M.

To construct pSL12 and pSL13, the following approach was used. PCRs were performed with (i) the upstream flanking primer and (ii) the primers P-3r and P-4r, respectively, on pUC19/5 as the template. The products were purified, treated with *Bam*HI, and cloned into pUC19 linearized by *Bam*HI and *Sma*I, giving pUC19/6 and pUC19/7, respectively. *Bam*HI-*Eco*RI fragments (about 800 and 880 bp, respectively) in which the *Eco*RI end was filled in by the Klenow fragment were then prepared. Finally, these fragments were ligated with pDH32M digested with *Bam*HI and *Sna*BI.

The products of another series of PCRs with pUC19/5 as the template were cloned into the unique *Eco*RV site of pBluescript II SK$^-$ (Stratagene). Plasmid pVL1 was obtained by using primers P-1 and P-4r (153-bp product), pVL4 was obtained with primer P-2 and the downstream flanking primer (475-bp product), and pVL5 was obtained with primers P-2 and P-4r (78-bp product). Furthermore, plasmid pVL6 was obtained with primers P-2cre and P-4r (86 bp), and pVL7 was obtained with primer P-2cre and the downstream flanking primer (485 bp). Recovery of the PCR products from these plasmids was done by digestion with *Bam*HI and *Hin*dIII. The purified fragments were cloned into pDH32M treated in the same way, giving plasmids pSL10, pSL14, pSL15, pSL16, and pSL17, respectively.

Plasmid pSK3 is a derivative of pBGW3 (46) with a *Sma*I-*Sna*BI deletion spanning the entire *licT* gene and the 5′ part of *licS* replaced by the erythromycin resistance gene (1.3-kb *Nci*I fragment) of pHP13 (17).

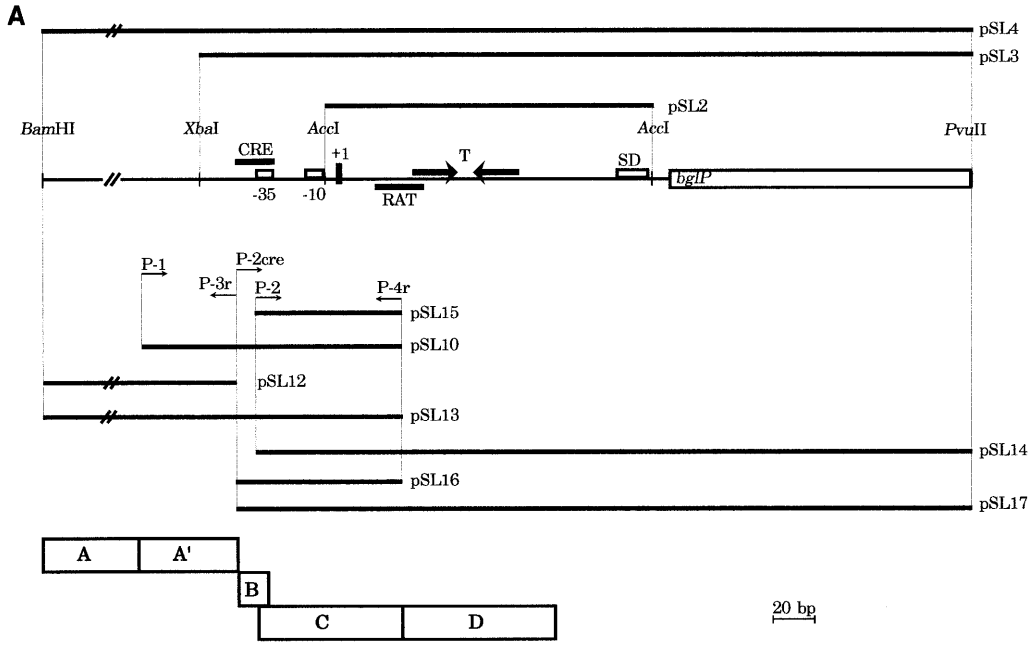
For complementation experiments, the *licT* gene was isolated from pBGW3 as a *Hin*dIII-*Nde*I fragment. The ends were filled in by the Klenow fragment and cloned into the *Hin*dIII site of plasmid pDG148 (45), also treated with the Klenow fragment of DNA polymerase I. In the resulting plasmid, pSG2, expression of *licT* is under control of the inducible promoter P_{spac}.

General genetic techniques. The standard transformation methods for *E. coli* and *B. subtilis* have been described previously (20, 39). Transformants were selected on solidified nutrient broth medium (1.5% [wt/vol] agar) supplemented with the relevant antibiotics as described above.

The isolation of plasmids from *E. coli* and chromosomal DNA from *B. subtilis* was performed according to standard procedures (21, 29).

DNA sequences were determined by the dideoxy-chain termination method (40) with plasmid DNA as the template and Sequenase kit version 2 (U.S. Biochemical Corp.).

Restriction enzymes, T4 DNA ligase, and other enzymes for DNA manipulation were purchased from commercial sources and were used according to the recommendations of the supplier. DNA fragments were purified from agarose gels by using the GeneClean II kit (Bio 101 Inc.). In general, plasmids which served as cloning vectors were dephosphorylated with shrimp phosphatase (U.S.



B

5'

GAAGTCTTTAAAAAAATTTAAACGGTAACGATTCATAACCTTGATTGGAAAAAATGCCTTTCAAGGTTTTTA

|-> P-1 *XbaI*

ATTACAATAATTTTTTATTGAAGTTAATACTAAGTTCAAATCTTTATGATTACTTTTGTTCTAGAAACGATAG

|-> P-2cre |-> P-2 *AccI*

TCAACACCAAAAATGAAAGCG**TTGACA**TCTCACGAATCTAGTGC**TAAAGT**ATACCT**A**CAAACAAATAAAAC

P-3r <-| CRE -35 -10 +1

P-4r <-|

GACCCGTCAGGA**TTGTTACTGCGAAAGCAGGCAAAACC**TAAATGGTGCAGGTGCAGGAGGAGACTCTCG

RAT Terminator

CAATCTGTGTCATTTAGGTTTTTTTATTCTCTTTTAGGAGGTGATGCAGCATTTGATACAAAAAAAGCTGGTG

AccI

AAAGGCGGCCACCTTTCATCCAGCAGTCCCCCCATAA**AAAGGAGG**TATACAC**ATG...**

RBS Met

FIG. 1. (A) Schematic drawing of the 1.1-kb *Bam*HI-*Hin*dIII *bglP* fragment with the positions and orientations of the primers and relevant restriction sites. The −35 and −10 boxes of the promoter, the transcriptional start point, and the Shine-Dalgarno region and the translation initiation codon are indicated. Bold letters represent the RAT sequence, and thick lines represent the conditional terminator. DNA fragments present in corresponding *lacZ* plasmids are shown. Boxes A, A′, B, C, and D are deduced from their potential involvement in the regulation of *bglP* synthesis. (B) Sequence of the regulatory region of *bglP*. Relevant elements of regulation are indicated. The 5′ ends of *bglP*-specific primers are shown.

Biochemical Corp.) after treatment with restriction enzymes and prior to ligation.

For the integration of *bglP′-lacZ* fusions, the corresponding plasmids were linearized with *Sca*I, allowing a double crossing over into the *amyE* site of the chromosome.

β-Galactosidase assay. *B. subtilis* cells containing *lacZ* fusions were grown in ASM and supplemented as indicated in Results. Samples (2 ml) were taken during the exponential phase and until 2.5 h after cells had entered the stationary phase unless stated otherwise. They were stored at −20°C until the enzyme assays were carried out as described previously (30). Experiments were performed at least in duplicate. The tables show representative results.

RESULTS

Regulation of *bglP* expression. A transcriptional fusion of the 1.1-kb *Bam*HI-*Pvu*II fragment of *bglP* (Fig. 1A) to the promoterless *lacZ* gene of plasmid pDH32M (26) was constructed and integrated into the *amyE* site of *B. subtilis* IS58. The synthesis of β-galactosidase in the resulting strain, BGW41, in the presence of different carbon sources is shown in Table 2. Expression was comparable when citrate or ribose was added. Moreover, *bglP* expression was induced over 60-fold in the presence of salicin plus ribose or citrate. The addition of 0.3% glucose led to a sharp decrease in β-galactosidase synthesis even in the presence of the inducer. Low concentrations of glucose resulted in repression of transcription during logarithmic growth in the presence of the inducer, but induction occurred after the onset of the stationary phase. Fructose, sucrose, and glucitol reduced the induction level two- to sevenfold, depending on the concentration used.

Sequence similarities indicate the presence of a ρ-independent termination structure (region D in Fig. 1A) which is partially overlapped by the RAT motif, the mRNA target of

TABLE 2. Effect of different carbon sources on expression of *bglP'-lacZ* fusion in strain BGW41[a]

Carbon source (%)[b]	β-Galactosidase activity (Miller units)			
	Logarithmic phase		Stationary phase	
	− Inducer	+ Inducer[c]	− Inducer	+ Inducer[c]
Citrate				
0.1	3.3	226	5.1	256
0.3	7.8	247	5.6	267
Ribose				
0.05	4.8	ND[d]	4.1	ND
0.1	4.6	231	3.8	247
0.2	4.4	205	3.8	246
Glucose				
0.05	0.8	13.7	4.7	114
0.1	1.3	8	29	121
0.2	ND	6	ND	4
0.3	1.2	7	0.9	5.3
Fructose				
0.05	2	60	14.9	163
0.1	2.5	62	2	43
0.2	2.1	59	2.1	37
Sucrose				
0.05	4.5	86	13.9	121
0.2	4.2	70	6.1	104
Glucitol				
0.1	2.5	93	1.8	95
0.3	2	82	1.3	99

[a] Cells were grown in ASM with the carbon source indicated and with or without an inducer (salicin). Samples were taken at an optical density of about 0.3 (logarithmic phase) and 1.5 h after cells entered the stationary phase.
[b] Final concentration (wt/vol).
[c] Salicin as an inducer was added to a final concentration of 0.2% (wt/vol).
[d] ND, not determined.

antitermination proteins (6, 22). Moreover, upstream from these sequences, a promoter probably recognized by the vegetative sigma factor σ^A was mapped (28) (Fig. 1B). It was verified that this promoter, characterized by primer extension (28), is the only promoter in front of the *bglP* gene. Strains BGW47 (*amyE*::pSL2) and BGW401 (*amyE*::pSL12) were constructed in order to show that no additional promoter, either internal or upstream, is present. β-Galactosidase activities of these strains were comparable to those of the empty *lacZ* vector plasmid pDH32M (strain BGW40) when grown in ASM (Table 3) (data not shown).

Effects of deletions of the regulatory region. For the determination of essential *cis*-acting sites, different parts upstream and/or downstream of the promoter were deleted by using restriction sites or by generating PCR products.

A deletion of region A and the 5′ part of region A′ (Fig. 1A) up to the *Xba*I site (in strain BGW46) had no effect compared with strain BGW41 (Table 3). Thus, all *cis*-acting sites responsible for the induction and catabolite repression of *bglP* expression seemed to be located downstream from the *Xba*I site.

Two strains lacking region D (BGW402 and BGW403) were investigated. Strain BGW403 showed a constitutive expression independent of the inducer, suggesting that the terminatorlike structure within region D is involved in the induction process of *bglP* expression by aryl-β-glucosides. However, β-galactosidase synthesis in BGW402 (region A was present) was about fivefold lower than that in BGW403 (lacking region A), indicating negative regulation by region A (Table 3 and Fig. 1A). This phenomenon was detectable only when region D was deleted (compare BGW41 and BGW46 in Table 3).

Expression of the *bglPΔ14'-lacZ* fusion of strain BGW406, in which regions A and A′ up to the −35 box of the promoter were deleted, showed a partial relief of glucose repression (Table 3). The putative catabolite responsive element (CRE) was truncated in these mutants, suggesting that this element is important for that kind of regulation (also described below). Synthesis of β-galactosidase was constitutive and did not undergo any regulatory mechanisms when the *lacZ* gene was under the control of region C (BGW407; Table 3). Therefore, initiation of transcription in region C did not require a positively acting element.

Influence of the *licT* gene product. In a previous work, it was shown that *bglP* maps in the vicinity of *licT* encoding an antitermination protein (28, 46). Moreover, it was proposed that the activity of LicT can be controlled by BglP (28). This led us to test whether expression of *bglP* is also dependent on the presence of *licT*. Therefore, plasmid pSL4 (with the entire regulatory region of *bglP*) was introduced into BGW10 (Δ*licTS*), giving BGW101. No β-galactosidase activity was

TABLE 3. Expression of different *bglP'-lacZ* fusions[a]

Strain	Relevant *bglP* fragment[b]	Presence of delimited regions[b]	β-Galactosidase activity (Miller units)[c]			
			0.1% Ribose	0.2% Salicin	0.3% Glucose	0.3% Glucose plus 0.2% salicin
BGW40[d]			0.4	ND[e]	ND	ND
BGW41	*Bam*HI→*Pvu*II	AA′BCD	3.8	250	0.9	5.3
BGW46	*Xba*I→*Pvu*II	(A′)[f]BCD	2.9	247	0.7	ND
BGW402	*Bam*HI→P-4r	AA′BC	64	14	7.4	ND
BGW403	P-1→P-4r	A′BC	354	89	36	ND
BGW406	P-2→*Pvu*II	(B)CD	4	336	4.1	65
BGW407	P-2→P-4r	(B)C	424	434	397	413
BGW408	P-2cre→P-4r	BC	328	111	51	48
BGW409	P-2cre→*Pvu*II	BCD	1.4	148	0.8	4.2

[a] Cells were grown in ASM supplemented with the indicated sugar substrates.
[b] See the legend to Fig. 1A for details.
[c] Samples were taken 1.5 h after cells entered the stationary phase at an optical density of about 1.
[d] BGW40 contained the promoterless *lacZ* gene of pDH32M integrated into the *amyE* site.
[e] ND, not determined.
[f] Parentheses indicate truncated regions.

TABLE 4. Control of *bglP′-lacZ* expression in *licT* mutants[a]

Strain	Relevant *bglP* fragment[b]	Presence of delimited region[b]	β-Galactosidase activity (Miller units)[c]	
			0.1% Ribose	0.2% Salicin
BGW101[d]	*Bam*HI→*Pvu*II	AA′BCD	0.7	0.4
BGW101(pSG2)[e]	*Bam*HI→*Pvu*II	AA′BCD	206	282
BGW104	P-2→*Pvu*II	(B)CD	0.3	0.1
BGW107	P-2→P-4r	(B)C	520	554

[a] Cells were grown in ASM supplemented with the indicated sugar substrates.
[b] See the legend to Fig. 1A for details. Parentheses indicate truncated regions.
[c] Samples were taken during the stationary phase at an optical density of about 1.
[d] The corresponding wild-type strain, BGW41, yielded 4 and 256 Miller units when grown with 0.1% ribose and 0.2% salicin, respectively.
[e] For induction of the P_{spac} promoter, IPTG was added to 0.5 mM.

measured when this strain was grown with or without an inducer (Table 4).

The phenotype of BGW101 could be altered in a complementation experiment. The presence of the replicative plasmid pSG2 (containing the *licT* gene) in BGW101 restored *bglP′-lacZ* expression when cultures were supplemented with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) (Table 4). The increased level even under noninducing conditions (0.1% ribose) might be due to overproduction of LicT. These results indicate an essential role of the *licT* gene product for the expression of the putative *bglPH* operon.

The possibility of cross talk with other antitermination proteins was investigated by using 0.05% sucrose as the sole carbon source in ASM. A low concentration of sucrose induces the *sacPA* operon, and the antitermination protein SacT is active (10). Since no increase in β-galactosidase activity was detected in BGW101, SacT cannot replace LicT to permit induction of *bglP* expression (data not shown). In contrast, other heterologous antiterminator-RAT pairs show significant cross-activity (6, 43).

Strain BGW104 (*licT*), lacking regions A and A′, exhibited no significant deviation in *bglP′-lacZ* expression compared with strain BGW101, underlining the strong dependence of BglP synthesis on LicT. These data indicate an efficient termination at an extremely stable hairpin structure. In the *licT* mutant BGW107 carrying region C (i.e., regions A, A′, B, and D were deleted), nearly the same pattern of expression (Table 4) was observed with respect to the isogenic wild-type BGW407 (Table 3).

Role of PTS components. The possible interaction of PTS components and LicT was studied by Le Coq and coworkers (28). Since a heterologous system, i.e., a *sacB′-lacZ* fusion, and a different genetic background were applied, we reinvestigated this kind of regulation by using derivatives of strain BGW41. Strain BGW415 was constructed carrying a *bglP′-lacZ* fusion at the *amyE* locus and a chromosomal deletion inactivating *bglP*. About 1,100 Miller units was measured during both the exponential and stationary phases, even in the absence of an inducer (Table 5). This high level of constitutive expression is probably due to the lack of BglP that cannot exert a negative effect on LicT.

The expression of the *bglP′-lacZ* fusion in the *ptsG* mutant BGW4103 corresponded completely to the data obtained with the isogenic wild type (data not shown). However, β-galactosidase synthesis in strain BGW420 (with the Δ*ptsGHI* mutation of GM273) was extremely low (less than 1 Miller unit; Table 5). Therefore, the general proteins of the PTS apparently exerted a positive regulatory effect on LicT activity. This strong regulation was suppressed by transformation of BGW420 with the replicative plasmid pSG2 (containing the *licT* gene). The respective strain was also grown in ASM with 0.1% of the non-PTS substrate glucitol plus 0.5 mM IPTG, and around 280 Miller units was obtained (Table 5). Since *licT* expression was not altered in a *ptsGHI* mutant, this result underlines the effect of the PTS on LicT activity (our unpublished results).

Glucose repression of *bglP* expression. The presence of 0.3% glucose resulted in a complete repression of *bglP′-lacZ* expression (Table 2). For the characterization of the CRE, a set of mutants were constructed. The putative CRE (5′-TGAAAGCGTTGACA-3′) was truncated by using primer P-2 (Fig. 1B), yielding BGW406 (with region D) and BGW407 (without region D). Expression independent of repressing amounts of glucose was obtained with strain BGW407, whereas strain BGW406 grown under inducing conditions showed only partial glucose resistance (Table 3). Exactly the putative CRE, but no additional upstream sequences, was present on relevant plasmids used for construction of BGW408 (lacking region D) and BGW409 (with region D). Since catabolite repression was exerted in strains BGW408 and BGW409 (Table 3), the CRE, which partially overlaps the −35 box, was suggested to be the key element in carbon catabolite repression of *bglP* expression. The motif deviated in 3 bases (positions 3, 9, and 11) with respect to an optimal operator that was described by Weickert and Chambliss (48).

Interestingly, there might be an additional mechanism or site of catabolite repression. Even in the absence of the CRE in strain BGW406, there was a weak but distinct influence of glucose. A fivefold repression of β-galactosidase activity was observed when cultures were supplemented with both 0.3% glucose and 0.2% salicin compared with under nonrepressing conditions (Table 3). In contrast to this, repression was about 50-fold when the CRE was present (BGW41; Table 3). Results indicating that repression was only six- to ninefold in strains BGW402, BGW403, and BGW408 (with region D deleted; Fig.

TABLE 5. Effects of mutations of PTS components on *bglP′-lacZ* expression[a]

Strain[b]	Relevant genotype	Carbon source	β-Galactosidase activity (Miller units)	
			Logarithmic phase	Stationary phase
BGW415	*bglP′-lacZ* Δ*bglP*::*spe*	0.1% Ribose	1,198	1,108
BGW420	*bglP′-lacZ* Δ*ptsGHI*::*erm*	0.1% Glucitol	0.6	0.7
BGW420(pSG2)[c]	*bglP′-lacZ* Δ*ptsGHI*::*erm*	0.1% Glucitol	279	274

[a] Cells were grown in ASM with the indicated carbon source. Samples were taken at an optical density of about 0.3 (logarithmic phase) and 1.5 h after cells entered the stationary phase.
[b] The respective data from the isogenic wild-type strain BGW41 can be taken from Table 2.
[c] For induction of the P_{spac} promoter, IPTG was added to 0.5 mM.

TABLE 6. Influence of *ccpA* and *ptsH1* mutations on catabolite repression[a]

Strain	Relevant genotype and *bglP* fragment[b]	β-Galactosidase activity (Miller units)			
		0.1% Ribose[c]	0.3% Glucose	0.2% Salicin	0.3% Glucose plus 0.2% salicin
BGW41	*bglP′-lacZ* (*Bam*HI→*Pvu*II) *ccpA*$^+$	4.2	1.1	256	3.8
BGW417	*bglP′-lacZ* (*Bam*HI→*Pvu*II) *ccpA*::Tn*917′* (Δ*erm*, *lacZ*)::*phl*	14	18	320	73
BGW403	*bglP*Δ*10′-lacZ* (P-1→P-4r) *ccpA*$^+$	369	32	95	ND[d]
BGW4171	*bglP*Δ*10′-lacZ* (P-1→P-4r) *ccpA*::Tn*917′* (Δ*erm*, *lacZ*)::*phl*	565	614	569	612
BGW4101	*bglP′-lacZ* (*Bam*HI→*Pvu*II) *ptsH*$^+$	6.6	ND	254	5.6
BGW4102	*bglP′-lacZ* (*Bam*HI→*Pvu*II) *ptsH1*	6.7	ND	381	122

[a] Cells were grown in ASM supplemented with the indicated sugar. Samples were taken 1.5 h after cells entered stationary phase at an optical density of about 1.
[b] See the legend to Fig. 1A.
[c] Ribose was included as control for nonrepressed enzyme synthesis.
[d] ND, not determined.

1A and Table 3) and that only BGW407 was completely glucose resistant strengthen this hypothesis (see Discussion).

Moreover, even 0.2% salicin led to repression of *bglP* expression when the RAT-terminator region was deleted (e.g., strains BGW403 and BGW408; Table 3 [also see Discussion]). On the other hand, 0.05% salicin did not repress β-galactosidase synthesis but did lead to a sharp decrease in enzyme synthesis at the onset of the stationary phase, probably due to limiting amounts of the substrate (data not shown).

A *trans*-acting factor involved in catabolite repression is the CcpA protein (19). Moreover, the PTS component HPr is involved in carbon catabolite repression of some catabolic genes (13). Expression of *bglP* in the respective mutant strains was investigated, and the results are shown in Table 6. Repression of enzyme synthesis in the *ccpA* mutant BGW417 was about 15-fold lower than that in the wild type. Enzyme synthesis was not altered by glucose in strain BGW4171 (*ccpA*) with regions A and D deleted. *bglP* expression in the *ptsH1* mutant BGW4102 was repressed only 3-fold by glucose, whereas expression in the isogenic wild-type strain BGW4101 was repressed 45-fold by glucose. Thus, CcpA and HPr seem to be involved in catabolite repression of BglP synthesis.

DISCUSSION

In this study, we have characterized the involvement of *cis*- and *trans*-acting elements in the regulation of *bglPH* expression.

After deletion of the region containing the terminatorlike structure of the *bglPH* system (region D in Fig. 1A), expression was independent of the availability of an inducer. Because expression was altered by the presence of region A, the presence of a negative-acting element is postulated (see Results). Whether this regulation is due to a short open reading frame found in region A (28) or is subject to a possible *trans*-acting factor that might bind to AT-rich sequences upstream of *bglP* (Fig. 1B) remains to be studied.

The data presented here strongly suggest that the *licT* gene encodes the antiterminator of the *bglPH* system (Table 4). LicT belongs to the BglG-SacY family of transcriptional antiterminators (46). A lack of the antitermination protein abolished β-galactosidase expression, presumably because of complete termination of transcription at a very stable hairpin structure (−30 kcal [−126 kJ]/mol). Thus, LicT seems to be a global-acting antitermination protein, since it is involved in the regulation of transcription of at least two genes, *bglPH* and *licS* (46). Moreover, the putative *bglPH* operon may be solely dependent on LicT, since neither cross talk with SacT (antiterminator of *sacPA*) nor cross talk with SacY (antiterminator of *sacB*), which is active in a PTS mutant (3), was observed. However, LicT is fully active at the terminator *bgl-t2* of the *E. coli bgl* operon (36).

In a previous study, both a positive effect of PTS components and a negative effect by the PTS-specific BglP protein on LicT activity were shown (28). We have presented evidence that positive regulation is mediated by the general PTS components (HPr and PtsI), since a *ptsG* mutation did not alter *bglP* expression. By increasing the number of copies of *licT* in BGW420 (*ptsGHI*), triggering a gene dosage effect, the dependence of *bglP* expression on the PTS was overcome. Thus, we speculate that the role of a PTS component(s) could be to facilitate the formation of a LicT dimer. It has been reported that BglG of *E. coli* is active when present as a dimer (2). Because of the high level of homology between antiterminators and similar regulatory mechanisms, it is imaginable that antitermination proteins of *B. subtilis* form dimers in order to bind to the RAT motif too. A higher copy number of LicT might lead to unspecifically formed dimers even in a Δ*ptsGHI* background. Since such a phenotype has not been reported before, we will study this in more detail in the future.

The expression of many catabolic genes is repressed when rapidly metabolizable carbon sources are available (15). In *B. subtilis*, *cis*-active sites which render expression of gene *amyE* (32) and of the *xyl* (25), *gnt* (31), *lic* (27), and *hut* (49) operons glucose resistant were identified. A partial deletion of a box homologous to those sequences resulted in glucose-resistant expression of *bglP′-lacZ* fusions (region B in Fig. 1A). This CRE partially overlaps the −35 box of the promoter of *bglP* (Fig. 1B). Thus, the relevant mechanism for catabolite repression seems to prevent the binding of the RNA polymerase. However, some CREs known to be active are also located downstream from the promoter, probably functioning by inhibition of transcription elongation of RNA polymerase (24).

An additional glucose repression mechanism apparently mediated via LicT can be postulated, since weak repression was measured when the CRE was deleted but the terminator structure was present (strain BGW406). Moreover, residual repression was observed in the *ccpA* mutant BGW417, indicating a second mechanism besides CRE-mediated catabolite repression. Since transcription of *licT* is not subject to carbon catabolite repression (our unpublished results) and multiple CREs cannot be found, the alteration of LicT activity by the PTS might be the reason for this phenomenon.

Since the sugar component of the aryl-β-glucoside salicin is glucose, *lacZ* expression was also repressed in a series of strains (with region D deleted) grown with 0.2% salicin (Table 3). Moreover, this result indicates that an intracellular signal probably formed by metabolization of glucose may be respon-

sible for carbon catabolite repression in *B. subtilis* (also described below). In *E. coli*, a high concentration of intracellular glucose does not exert such an effect, but glucose uptake and, consequently, (i) low levels of cAMP synthesis and (ii) inducer exclusion have been shown to be crucial mechanisms in catabolite repression (34).

The CcpA protein is involved in catabolite repression of *B. subtilis* (8). Recently, a model has suggested that CcpA and HPr act together, possibly by protein-protein interaction, to bind DNA and repress transcription (13). An HPr mutant strain which lacks the second phosphorylation site (serine 46) led to glucose-insensitive expression of several genes (13, 27). The phosphorylation of HPr at Ser-46 depends on an ATP-dependent, fructose-1,6-diphosphate-activated protein kinase (14, 37). Since only HPr (Ser-P) has been shown to interact with CcpA, it has been suggested that HPr represents the key regulatory element in catabolite repression in *B. subtilis* (12, 23). The expression of *bglP′-lacZ* fusions in *ccpA* or *ptsH1* mutants was glucose resistant, suggesting their involvement in carbon catabolite repression of *bglP* and supporting the model mentioned above.

In conclusion, the *bglP-bglH* system is subject to both termination and antitermination regulation and carbon catabolite repression—a dual regulation that has also been reported for the *E. coli bgl* operon (38, 41).

ACKNOWLEDGMENTS

We are grateful to M. Steinmetz and D. Le Coq for the generous contribution of strains and plasmids and helpful discussions and J. Stülke for reading the manuscript and for discussions. A. Tschirner is acknowledged for excellent technical assistance, and S. Gertz is acknowledged for the construction of pSG2. We acknowledge J. Deutscher and M. Arnaud for the gift of strains and W. Hillen for communicating results prior to publication.

This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. S.K. is a fellow of the DECHEMA e.V.

REFERENCES

1. **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.
2. **Amster-Choder, O., and A. Wright.** 1992. Modulation of the dimerization of a transcriptional antiterminator protein by phosphorylation. Science **257:** 1395–1398.
3. **Arnaud, M., P. Vary, M. Zagorec, A. Klier, M. Debarbouille, P. Postma, and G. Rapoport.** 1992. Regulation of the *sacPA* operon of *Bacillus subtilis*: identification of phosphotransferase system components involved in SacT activity. J. Bacteriol. **174:**3161–3170.
4. **Aymerich, S., G. Gonzy-Tréboul, and M. Steinmetz.** 1986. 5′-noncoding region *sacR* is the target of all identified regulation affecting the levansucrase gene in *Bacillus subtilis*. J. Bacteriol. **166:**993–998.
5. **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.
6. **Aymerich, S., and M. Steinmetz.** 1992. Specificity determinants and structural features in the RNA target of the bacterial antiterminator proteins of the BglG/SacY family. Proc. Natl. Acad. Sci. USA **89:**10410–10414.
7. **Bolivar, F., R. L. Rodriguez, P. J. Greener, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–133.
8. **Chambliss, G. H.** 1993. Carbon source-mediated catabolite repression, p. 213–219. *In* A. L. Sonenshein, J. A. Hoch, and R. Losick (ed.), *Bacillus subtilis* and other gram-positive bacteria: biochemistry, physiology, and molecular genetics. American Society for Microbiology, Washington, D.C.
9. **Crutz, A. M., M. Steinmetz, S. Aymerich, R. Richter, and D. Le Coq.** 1990. Induction of levansucrase in *Bacillus subtilis*: an antitermination mechanism negatively controlled by the phosphotransferase system. J. Bacteriol. **172:** 1043–1050.
10. **Debarbouille, M., M. Arnaud, A. Fouet, A. Klier, and G. Rapoport.** 1990. The *sacT* gene regulating the *sacPA* operon in *Bacillus subtilis* shares strong homology with transcriptional antiterminators. J. Bacteriol. **172:**3966–3973.
11. **Débarbouillé, M., I. Martin-Verstraete, M. Arnaud, A. Klier, and G. Rapoport.** 1991. Positive and negative regulation controlling expression of the *sac* genes in *Bacillus subtilis*. Res. Microbiol. **142:**757–764.
12. **Deutscher, J., E. Küster, U. Bergstedt, V. Charrier, and W. Hillen.** 1995. Protein kinase-dependent HPr/CcpA interaction links glycolytic activity to carbon catabolite repression in gram-positive bacteria. Mol. Microbiol. **15:** 1049–1053.
13. **Deutscher, J., J. Reizer, C. Fischer, A. Galinier, M. H. Saier, Jr., and M. Steinmetz.** 1994. Loss of protein kinase-catalyzed phosphorylation of HPr, a phosphocarrier protein of the phosphotransferase system, by mutation of the *ptsH* gene confers catabolite repression resistance to several catabolic genes of *Bacillus subtilis*. J. Bacteriol. **176:**3336–3344.
14. **Deutscher, J., and M. H. Saier, Jr.** 1983. ATP-dependent, protein kinase-catalyzed phosphorylation of a seryl residue in HPr, the phosphoryl carrier protein of the phosphotransferase system in *Streptococcus pyogenes*. Proc. Natl. Acad. Sci. USA **80:**6790–6794.
15. **Fisher, S. H., and A. L. Sonenshein.** 1991. Control of carbon and nitrogen metabolism in *Bacillus subtilis*. Annu. Rev. Microbiol. **45:**107–135.
16. **Glaser, P., F. Kunst, M. Arnaud, M. P. Coudart, W. Gonzales, M. F. Hullo, M. Ionescu, B. Lubochinsky, L. Marcelino, I. Moszer, E. Presecan, M. Santana, E. Schneider, J. Schweizer, and A. Vertes.** 1993. *Bacillus subtilis* genome project: cloning and sequencing of the 97kb region from 325 degrees to 333 degrees. Mol. Microbiol. **10:**371–384.
17. **Haima, P., S. Bron, and G. Venema.** 1987. The effect of restriction on shotgun cloning and plasmid stability in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **209:**335–342.
18. **Hall, B. G., and L. Xu.** 1992. Nucleotide sequence, function, activation, and evolution of the cryptic *asc* operon of *Escherichia coli* K12. Mol. Biol. Evol. **9:**688–704.
19. **Henkin, T. M., F. J. Grundy, W. L. Nicholson, and G. H. Chambliss.** 1991. Catabolite repression of alpha-amylase gene expression in *Bacillus subtilis* involves a *trans*-acting gene product homologous to the *Escherichia coli lacI* and *galR* repressors. Mol. Microbiol. **5:**575–584.
20. **Hoch, J. A.** 1991. Genetic analysis in *Bacillus subtilis*. Methods Enzymol. **204:**305–320.
21. **Holmes, P. S., and M. Quigley.** 1981. A rapid boiling method for the preparation of bacterial plasmids. Anal. Biochem. **114:**187–193.
22. **Houman, F., M. R. Diaz-Torres, and A. Wright.** 1990. Transcriptional antitermination in the *bgl* operon of *E. coli* is modulated by a specific RNA binding protein. Cell **62:**1153–1163.
23. **Hueck, C. J., and W. Hillen.** 1995. Catabolite repression in *Bacillus subtilis*: a global regulatory mechanism for gram-positive bacteria? Mol. Microbiol. **15:**395–401.
24. **Hueck, C. J., W. Hillen, and M. H. Saier.** 1994. Analysis of a *cis*-active sequence mediating catabolite repression in gram-positive bacteria. Res. Microbiol. **145:**503–518.
25. **Jacob, S., R. Allmannsberger, D. Gärtner, and W. Hillen.** 1991. Catabolite repression of the operon for xylose utilization from *Bacillus subtilis* W23 is mediated at the level of transcription and depends on a *cis* site in the *xylA* reading frame. Mol. Gen. Genet. **229:**189–196.
26. **Kraus, A., C. Hueck, D. Gärtner, and W. Hillen.** 1994. Catabolite repression of the *Bacillus subtilis xyl* operon involves a *cis* element functional in the context of an unrelated sequence, and glucose exerts additional *xylR*-dependent repression. J. Bacteriol. **176:**1738–1745.
27. **Krüger, S., J. Stülke, and M. Hecker.** 1993. Catabolite repression of β-glucanase synthesis in *Bacillus subtilis*. J. Gen. Microbiol. **139:**2047–2054.
28. **Le Coq, D., C. Lindner, S. Krüger, M. Steinmetz, and J. Stülke.** 1995. New β-glucoside (*bgl*) genes in *Bacillus subtilis*: the *bglP* gene product has both transport and regulatory functions, similar to those of BglF, its *Escherichia coli* homolog. J. Bacteriol. **177:**1527–1535.
29. **Meade, H. M., S. R. Long, G. B. Ruvkun, S. E. Brown, and F. M. Ausubel.** 1982. Physical and genetic characterization of symbiotic and auxotrophic mutants of *Rhizobium meliloti* induced by transposon Tn*5* mutagenesis. J. Bacteriol. **149:**114–122.
30. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
31. **Miwa, Y., and Y. Fujita.** 1990. Determination of the *cis* sequence involved in catabolite repression of the *Bacillus subtilis gnt* operon: implication of a consensus sequence in catabolite repression in the genus *Bacillus*. Nucleic Acids Res. **18:**7049–7053.
32. **Nicholson, W. L., and G. H. Chambliss.** 1985. Isolation and characterization of a *cis*-acting mutation conferring catabolite repression resistance to α-amylase synthesis in *Bacillus subtilis*. J. Bacteriol. **161:**875–881.
33. **Parker, L. L., and B. G. Hall.** 1990. Characterization and nucleotide sequence of the cryptic *cel* operon of *E. coli* K 12. Genetics **124:**455–471.
34. **Postma, P. W., J. W. Lengeler, and G. R. Jacobson.** 1993. Phosphoenolpyruvate:carbohydrate phosphotransferase system of bacteria. Microbiol. Rev. **57:**543–594.
35. **Prasad, I., B. Young, and S. Schaefler.** 1973. Genetic determination of the constitutive biosynthesis of phospho-β-glucosidase A in *Escherichia coli* K-12. J. Bacteriol. **114:**909–915.
36. **Rak, B.** 1993. Personal communication.

37. **Reizer, J., M. J. Novotny, W. Hengstenberg, and M. H. Saier, Jr.** 1984. Properties of ATP-dependent protein kinase from *Streptococcus pyogenes* that phosphorylates a seryl residue in HPr, a phosphocarrier protein of the phosphotransferase system. J. Bacteriol. **160:**333–340.
38. **Reynolds, A. E., S. Mahadevan, S. F. J. Le Grice, and A. Wright.** 1986. Enhancement of bacterial gene expression by insertion elements or by mutation in a CAP-cAMP binding site. J. Mol. Biol. **191:**85–95.
39. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
40. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
41. **Schnetz, K., and B. Rak.** 1990. β-Glucoside permease represses the *bgl* operon of *Escherichia coli* by phosphorylation of the antiterminator protein and also interacts with glucose-specific enzyme III, the key element in catabolite control. Proc. Natl. Acad. Sci. USA **87:**5074–5078.
42. **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.
43. **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.
44. **Steinmetz, M., and R. Richter.** 1994. Plasmids designed to alter the antibiotic resistance expressed by insertion mutations in *Bacillus subtilis* through in vivo recombination. Gene **142:**79–83.
45. **Stragier, P., C. Bonamy, and C. Karmazyn-Campelli.** 1988. Processing of a sporulation sigma factor in *Bacillus subtilis*: how morphological structure could control gene expression. Cell **52:**697–704.
46. **Stülke, J.** 1993. Mechanismen der Regulation der β-Glucanase Synthese in *Bacillus subtilis*. Ph.D. thesis. Ernst-Moritz-Arndt University, Greifswald, Germany.
47. **Stülke, J., R. Hanschke, and M. Hecker.** 1993. Temporal activation of β-glucanase synthesis in *Bacillus subtilis* is mediated by the GTP pool. J. Gen. Microbiol. **139:**2041–2045.
48. **Weickert, M. J., and G. H. Chambliss.** 1990. Site-directed mutagenesis of a catabolite repression operator sequence in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **87:**6238–6242.
49. **Wray, L. V., Jr., F. K. Pettengill, and S. H. Fisher.** 1994. Catabolite repression of the *Bacillus subtilis hut* operon requires a *cis*-acting site located downstream of the transcription initiation site. J. Bacteriol. **176:**1894–1902.
50. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103–119.
51. **Zhang, J. K., and A. Aronson.** 1994. A *Bacillus subtilis bglA* gene encoding phospho-β-glucosidase is inducible and closely linked to a NADH dehydrogenase-encoding gene. Gene **140:**85–90.