JOURNAL OF BACTERIOLOGY, Jan. 1990, p. 86–93
0021-9193/90/010086-08$02.00/0

Vol. 172, No. 1

# The *spoIIJ* Gene, Which Regulates Early Developmental Steps in *Bacillus subtilis*, Belongs to a Class of Environmentally Responsive Genes

CHRISTOPHE ANTONIEWSKI,[1] BRUNO SAVELLI,[2] AND PATRICK STRAGIER[2]*

*Institut de Microbiologie, Bâtiment 409, Université Paris-Sud, 91405 Orsay Cedex,[1] and Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris,[2] France*

Received 15 September 1989/Accepted 29 September 1989

**The *Bacillus subtilis spoIIJ* locus is defined by a Tn*917* insertion which leads to an oligosporogenous phenotype. Here we show that this mutation severely decreases transcription of *spoIIA*, *spoIIE*, and *spoIIG*, three operons involved in asymmetric septation, the earliest morphological event of sporulation. A 14.3-kilobase region overlapping the site of the *spoIIJ*::Tn*917* insertion was cloned and the exact location of the *spoIIJ* gene was defined with various integrative plasmids carrying subfragments of that region. DNA sequencing established that *spoIIJ* is a monocistronic locus encoding a 606-amino-acid polypeptide which contains a canonical "transmitter" domain, indicating that *spoIIJ* is a new member of the "sensor" class of signal-transducing systems in bacteria. Thus, *spoIIJ*, which is transcribed during vegetative growth, presumably under the control of $\sigma^H$, encodes a protein that could interact with major regulators of early sporulation stages, such as Spo0A and/or Spo0F.**

Sporulation of *Bacillus subtilis* is induced by starvation for either a carbon, a nitrogen, or a phosphorus source. The first recognizable morphological step of this developmental process is the synthesis of an asymmetric septum at one pole of the cell (reviewed in reference 23). This abnormal septation, which segregates the two chromosomes issued from the last round of replication into two unequal compartments, takes place about 60 to 90 min after the end of exponential growth in liquid medium at 37°C. Mutations in the *spoIIA* and *spoIIE* operons lead to aberrant structures often characterized by multiple septa, excess membrane synthesis, and deposition of cell wall inside the septa (23). These mutations also block the processing of the inactive precursor of $\sigma^E$, a sigma factor which plays a major role in transcription specificity after the cell has divided into two compartments (35, 37). It has been proposed that activation of the *spoIIGA* product, the presumptive processing enzyme (35), is triggered by the sporulation septum itself, which in turn activates pro-$\sigma^E$, the *spoIIGB* product (18, 35). Interestingly, the three operons *spoIIA*, *spoIIE*, and *spoIIG* are turned on simultaneously about 30 min after the onset of sporulation, and their transcription depends on the products of the *spo0* genes, suggesting that their expression could be coordinated by a common regulatory mechanism (7, 12, 16, 35).

Since some *spo0* genes are transcribed during exponential growth (4, 40), it can be conjectured that their products respond to the metabolic status of the cell and relay this information by controlling expression of early *spo* genes, such as some stage II genes. Sequencing of *spo0A* (9) and *spo0F* (36) has revealed that their products belong to a family of widespread procaryotic proteins, most of which activate transcription of various regulons in response to specific environmental signals. These proteins are part of a two-component system, and their activity is modulated by a second protein acting as a "sensor" and a "transmitter" of some metabolic stimuli (27). It is thus expected that among the early *spo* gene products there should be some protein(s) interacting with Spo0A and/or Spo0F in response to nutrient deprivation. The sequence of the *spoIIJ* gene reported in this article suggests that its product could play such a role.

(A preliminary account of these results [including the similarity of SpoIIJ with the sensor class of the two-component systems] was presented at the 10th International Spores Conference, Woods Hole, Mass., 23 to 27 March 1988, abstr. no. 26.)

## MATERIALS AND METHODS

**Bacterial strains and media.** Most of the experiments were carried out with *B. subtilis* JH642 *trpC2 pheA1*. Other strains were IS233 (JH642 *spo0H* Δ*Hin*dIII) (38), KS19 (*spoIIJ*::Tn*917*ΩHU19) (28) (both provided by S. Cutting), and strain MO434 (JH642 *ptsI*::*cat*), provided by G. Gonzy-Tréboul. Transformation of *B. subtilis* was done as described before (2). For all sporulation experiments, *B. subtilis* strains were grown in DS medium (30). Chloramphenicol (5 μg/ml) and a mixture of erythromycin (0.5 μg/ml) and lincomycin (12.5 μg/ml) were added when necessary.

Plasmid constructions were performed with the *Escherichia coli* strains DH5α and TG1. *E. coli* bacteria were grown in LB medium (19) in the presence of the appropriate antibiotics (ampicillin [50 μg/ml] or tetracycline [10 μg/ml]).

**Shuttle vectors.** Marker replacement experiments (5) with chromosomally inserted transposon Tn*917* were made by using plasmids pTV21Δ2 (41) and pTV55 (42) linearized with *Xba*I. Plasmids able to integrate by a single recombination event into the *B. subtilis* chromosome were derivatives of pJH101 (8) or pDG271, a plasmid constructed in our lab by introducing the *cat* marker from pC194 between the *Eco*RI and *Hin*dIII sites of the versatile cloning vector pJRD184 (13).

**Construction of *lacZ* fusions.** The *spoIIA*- and *spoIIE-lacZ* fusions have already been described (35). The *spoIIG-lacZ* fusion was constructed by subcloning a 518-base-pair (bp) *Hpa*II fragment containing the *spoIIG* promoter in the *Acc*I site of pUC8 and then cloning the resulting *Hin*dIII-*Bam*HI fragment in pDG268 upstream of a promoterless *lacZ* gene

* Corresponding author.

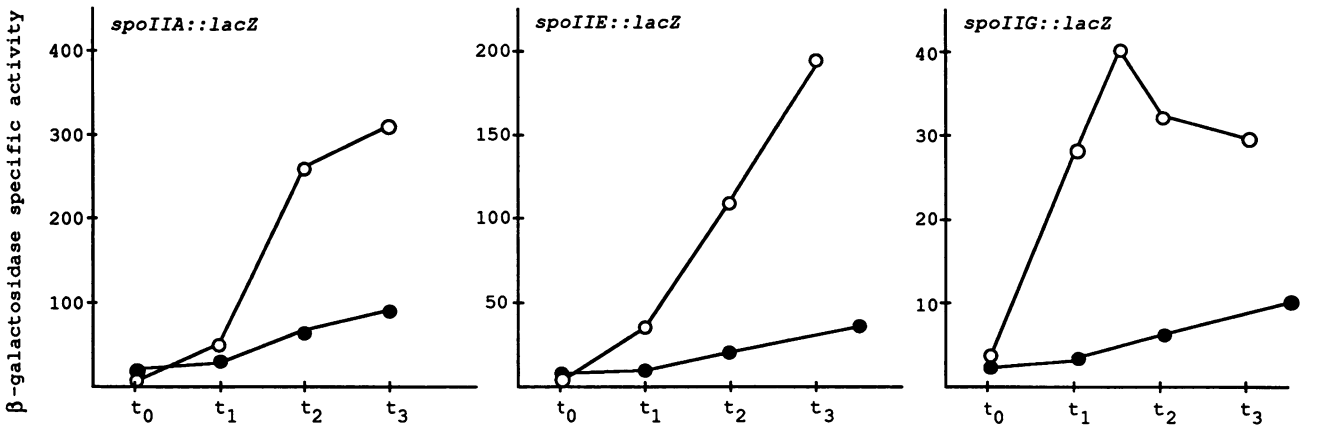


FIG. 1. Effect of the *spoIIJ*::Tn*917* mutation on *spoIIA*-, *spoIIE*-, and *spoIIG-lacZ* expression. The specific activity of β-galactosidase in strain JH642 (*spoIIJ*$^+$) (○) or in strain MO845 (*spoIIJ*::Tn*917*) (●) carrying the indicated *lacZ* fusion was monitored after cells were induced to sporulate by exhaustion of DS medium. $t_n$ indicates hours after the onset of sporulation.

which uses the translational signals of the *B. subtilis spoVG* gene. This plasmid was constructed in our lab by introducing at the original cloning sites of pDH32 (32) an *Eco*RI-*Hin*dIII-*Bam*HI polylinker and by replacing the pBR322 backbone with the equivalent region of pJRD184. The *spoIIJ-lacZ* fusion was constructed by cloning a 660-bp *Sac*I-*Nsi*I fragment containing the *spoIIJ* promoter in pUC19 and then recloning the resulting *Eco*RI-*Hin*dIII fragment in pDG268. Both *spoIIG-* and *spoIIJ-lacZ* fusions were introduced into the chromosome after linearization of the pDG268 derivative plasmids. β-Galactosidase specific activity was measured on sonicated extracts as described previously (35) and expressed as nanomoles of 2-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of protein.

**Sequencing procedures.** Most of the nucleotide sequence was obtained by using the in vivo deletion procedure devised by Ahmed (1) after cloning two overlapping fragments of the *spoIIJ* locus in the pAAZ718 and pAAZ719 plasmids, purchased from Gold Biotechnology Ltd. (22). The sequence was completed by subcloning restriction fragments in the Bluescript phagemid (33) or in the pTZ18R/18U and pTZ19R/19U phagemids (Pharmacia). In all cases, single-stranded DNA was prepared and subjected to the dideoxy sequencing procedure (29) as subsequently modified (3). Sequencing outside of the *Cla*I-*Sph*I fragment shown in Fig. 3 was done mostly on one strand only, and the data are not given here.

## RESULTS

**A mutation in *spoIIJ* blocks expression of other stage II genes.** Sandman et al. isolated a collection of random insertions of the Tn*917* transposon into the *B. subtilis* chromosome that led to an asporogenous phenotype (28). Some of these insertions could not be correlated to known loci and defined new *spo* genes, such as the HU19 insertion which inactivated the *spoIIJ* gene. This gene was mapped by transduction around 120° on the *B. subtilis* chromosome and was found to be different from other *spo* markers located in the same region. The Tn*917*ΩHU19 insertion mutant was described to still produce about 30% of the wild-type level of heat-resistant spores (28). We confirmed these results after introduction of this mutation into another genetic background; transformation of strain JH642 with chromosomal DNA from strain KS19 and selection for erythromycin resistance created strain MO845, which sporulated with 10 to 30% efficiency. This oligosporogenous phenotype could be corrected by transformation with chromosomal DNA from a strain carrying a chloramphenicol resistance marker inserted in the *ptsI* gene; 23% of the chloramphenicol-resistant transformants simultaneously became erythromycin sensitive and sporulation proficient. This genetic linkage confirmed and defined more precisely the location of *spoIIJ* at 120°, very near *ptsI*.

As a first approach to define the physiological role of the *spoIIJ* product in the sporulation process, we studied the expression of the *spoIIA*, *spoIIE*, and *spoIIG* operons in a *spoIIJ* background. For that purpose, transcriptional fusions of the *lacZ* gene with the *spoIIA*, *spoIIE*, and *spoIIG* promoters were introduced into strains JH642 and MO845. It should be noted that the fusions with *spoIIA* and *spoIIE* were made through a Campbell-like recombination event, which disrupted these loci and led to a strong stage II blockage. Nevertheless, these fusions could be used to follow *spoIIA* and *spoIIE* expression until stage II, the ensuing transcriptional shut-off being lost in these mutants (35). Conversely, the *spoIIG-lacZ* fusion, which was integrated at the *amy* locus, left an intact *spoIIG* operon. In each case the presence of the *spoIIJ* insertional mutation led to a severe decrease in β-galactosidase synthesis, with only 18 to 25% activity remaining at $t_2$ (2 h after initiation of sporulation) (Fig. 1). Thus, transcription of the *spoIIA*, *spoIIE*, and *spoIIG* operons in a *spoIIJ* mutant seems to reflect the proportion of sporulating bacteria, suggesting that it is abolished in cells in which the absence of the *spoIIJ* product has blocked sporulation.

**Cloning of the *spoIIJ* locus.** In order to get some insight on its function, we decided to clone and sequence the *spoIIJ* locus. We took advantage of the transposon inserted in *spoIIJ* and followed the strategy devised by Youngman and co-workers to clone DNA fragments adjacent to Tn*917* insertions (41). Transformation of strain MO845 with linearized pTV21Δ2 and selection for chloramphenicol resistance introduced an *E. coli* replicon into the *B. subtilis* chromosome at the *spoIIJ* locus. Submitting chromosomal DNA from that strain to the action of various restriction enzymes allowed us to recover, after ligation and transformation of *E. coli*, several overlapping fragments located upstream of the *spoIIJ* Tn*917* insertion and one fragment located downstream of that insertion (Fig. 2).

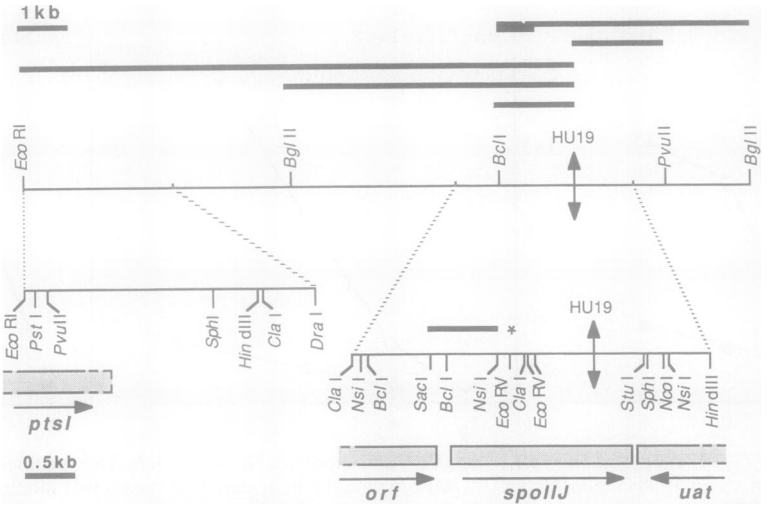


FIG. 2. Physical map of the *spoIIJ* locus. In the upper part are shown the various fragments that were cloned. Only the restriction sites involved in these cloning steps are indicated. The thicker bar shows a *Bcl*I-*Eco*RV fragment that was used to clone over the site of the HU19 insertion (indicated by the double-headed vertical arrow). In the lower part are shown two regions that were characterized in detail (note the different scales). The coding regions are indicated as stippled boxes. The asterisk points to a *Sac*I-*Nsi*I fragment that was used for construction of a *spoIIJ-lacZ* fusion.

These plasmids were then used to transform strain JH642 by selection for chloramphenicol resistance. Campbell-like integration of the plasmid containing a 1.4-kilobase (kb) fragment produced by digestion with *Bcl*I led to a typical SpoIIJ$^-$ phenotype, while bacteria remained completely Spo$^+$ after integration of the plasmid containing a 5.5-kb fragment produced by digestion with *Bgl*II. These results indicate that one end of the *spoIIJ* locus is located between these *Bcl*I and *Bgl*II sites (24). On the other side, integration of the plasmid carrying a 1.8-kb fragment produced by digestion with *Pvu*II did not lead to an oligosporogenous phenotype, indicating that the other end of the *spoIIJ* locus is contained within that fragment.

In order to obtain a fragment overlapping the site of the Tn*917*ΩHU19 insertion, we subcloned into an integrative vector a 0.6-kb *Bcl*I-*Eco*RV fragment internal to the *spoIIJ* locus and located upstream of the transposon insertion (Fig. 2). After transformation of strain JH642 with this plasmid, we used an approach similar to the one described above to clone an intact 4.9-kb fragment extending to a *Bgl*II site located 3.5 kb downstream of the site of the HU19 insertion. This fragment was subsequently used for sequencing and reconstructing in vitro a complete *spoIIJ* gene.

Due to the genetic linkage of *spoIIJ* with *ptsI*, we suspected that part of the *ptsI* locus could be carried by the larger cloned chromosomal fragment which extends to an *Eco*RI site located 10.8 kb upstream of the HU19 insertion. To investigate this possibility, we subcloned the distal part of this region as a 2.9-kb *Eco*RI-*Dra*I fragment. Its physical structure was characterized (Fig. 2) and found to fit perfectly with the map of the downstream part of the *ptsI* locus (11; G. Gonzy-Tréboul, personal communication). Since the *ptsI* gene is estimated to extend about 0.8 kb downstream of the *Eco*RI site (11), a 0.55-kb *Eco*RI-*Rsa*I fragment was cloned into an integrative vector, and the resulting plasmid was recombined into the chromosome of strain JH642. All the chloramphenicol-resistant transformants were found to be unable to grow on mannitol as the sole carbon source, indicating that the *ptsI* gene had been disrupted in these clones. Thus, the *Eco*RI fragment cloned from the Tn*917*ΩHU19 insertion overlaps the region already cloned by Gonzy-Tréboul et al. in their study of the *pts* locus (11) and the *spoIIJ* transposon insertion appears to be located about 10 kb downstream of *ptsI*.

**Nucleotide sequence of the *spoIIJ* locus.** To delineate the borders of the *spoIIJ* locus on both sides of the HU19 insertion, various fragments were subcloned into integrative vectors, introduced by transformation into strain JH642, and checked for their effect on the Spo phenotype of the resulting transformants. These experiments indicated that the *spoIIJ* locus does not extend outside of a 3.5-kb *Cla*I-*Hin*dIII fragment (Fig. 2), which was then submitted to nucleotide sequence analysis. The exact location of the HU19 insertion was determined by sequencing both junctions of the Tn*917* transposon with chromosomal DNA in fragments cloned from the strain carrying the Tn*917*ΩHU19 mutation. The transposon was found to have inserted into a 606-codon open reading frame which defines the *spoIIJ* gene (Fig. 3). The *Bcl*I site, known from the experiments described in the previous section to be located within the *spoIIJ* transcriptional unit, appears immediately upstream of a strong putative ribosome-binding site preceding the open reading frame disrupted by the transposon.

The *spoIIJ* gene is preceded at 163 bp by another open reading frame which starts outside of the sequenced region and extends for more than 268 codons. Disruption of this gene with an internal restriction fragment which would eliminate the last 42 amino acids of its product did not lead to any recognizable phenotype. This open reading frame is followed by an inverted repeat that could be a transcription termination signal (Fig. 3). Since our sequencing data, together with the results obtained above with the integrative plasmids, indicate that *spoIIJ* transcription starts in the *Cla*I-*Bcl*I interval (Fig. 2), this upstream open reading frame cannot be part of the *spoIIJ* operon.

Only 10 bp downstream of *spoIIJ* we found on the other strand a third open reading frame extending for more than 246 codons. This reading frame partially overlaps an inverted repeat which could be used as a transcription termination signal for *spoIIJ* (Fig. 3). Attempts to disrupt this gene with an integrative plasmid carrying an *Sph*I-*Nsi*I fragment that would have deleted the last 30 codons were

```
GAGCTCGAAAAAAAACACAGTAAAAAATAAAAAACAGGGTGCACAACTAAAAGATTGTGTGCCCTTTCTTTTATTCAAAAATTGACGTTCACCATAAGAATAGAAGGAGAATACTCAT   118

TTTCTAGCGAATCATACTAGGTAAAAGTCAATCTGTATATGTCGAAACACGATGATCATGCAAAGGAGGGATTCT GTG GAA CAG GAT ACG CAG CAT GTT AAA CCA CTT   226
                                                               Met Glu Gln Asp Thr Gln His Val Lys Pro Leu    11

CAA ACA AAA ACC GAT ATT CAT GCA GTC TTG GCC TCT AAT GGA CGC ATC ATT TAT ATA TCT GCC AAC TCC AAA CTG CAT TTG GGC TAT CTC   316
Gln Thr Lys Thr Asp Ile His Ala Val Leu Ala Ser Asn Gly Arg Ile Ile Tyr Ile Ser Ala Asn Ser Lys Leu His Leu Gly Tyr Leu    41

CAA GGA GAG ATG ATC GGA TCA TTC CTC AAA ACG TTT CTG CAT GAG GAA GAC CAA TTT TTG GTT GAA AGC TAT TTT TAT AAT GAA CAT CAT   406
Gln Gly Glu Met Ile Gly Ser Phe Leu Lys Thr Phe Leu His Glu Glu Asp Gln Phe Leu Val Glu Ser Tyr Phe Tyr Asn Glu His His    71

CTG ATG CCG TGC ACC TTT CGT TTT ATT AAA AAA GAT CAT ACG ATT GTG TGG GTG GAG GCT GCG GTA GAA ATT GTT ACG ACA AGA GCT GAG   496
Leu Met Pro Cys Thr Phe Arg Phe Ile Lys Lys Asp His Thr Ile Val Trp Val Glu Ala Ala Val Glu Ile Val Thr Thr Arg Ala Glu   101

CGG ACA GAA CGG GAA ATC ATT TTG AAA ATG AAG GTT CTT GAA GAA GAA ACA GGC CAT CAA TCC CTA AAC TGC GAA AAA CAT GAA ATC GAA   586
Arg Thr Glu Arg Glu Ile Ile Leu Lys Met Lys Val Leu Glu Glu Glu Thr Gly His Gln Ser Leu Asn Cys Glu Lys His Glu Ile Glu   131

CCT GCA AGC CCG GAA TCG ACT ACA TAT ATA ACG GAT GAT TAT GAA CGG TTG GTT GAA AAT CTC CCG AGT CCG CTA TGC ATC AGT GTC AAA   676
Pro Ala Ser Pro Glu Ser Thr Thr Tyr Ile Thr Asp Asp Tyr Glu Arg Leu Val Glu Asn Leu Pro Ser Pro Leu Cys Ile Ser Val Lys   161

GGC AAG ATC GTC TAT GTA AAC AGC GCG ATG CTT TCA ATG CTG GGA GCC AAA AGC AAG GAT GCT ATT ATT GGT AAA TCG TCC TAT GAA TTT   766
Gly Lys Ile Val Tyr Val Asn Ser Ala Met Leu Ser Met Leu Gly Ala Lys Ser Lys Asp Ala Ile Ile Gly Lys Ser Ser Tyr Glu Phe   191

ATT GAA GAA GAA TAT CAT GAT ATC GTG AAA AAC AGG ATT ATA CGA ATG CAA AAA GGA ATG GAA GTC GGA ATG ATT GAA CAG ACG TGG AAA   856
Ile Glu Glu Glu Tyr His Asp Ile Val Lys Asn Arg Ile Ile Arg Met Gln Lys Gly Met Glu Val Gly Met Ile Glu Gln Thr Trp Lys   221

AGG CTT GAT GGC ACA CCT GTT CAT TTA GAA GTG AAA GCA TCC CCG ACC GTC TAC AAA AAC CAG CAG GCT GAG CTG CTG CTG CTG ATC GAT   946
Arg Leu Asp Gly Thr Pro Val His Leu Glu Val Lys Ala Ser Pro Thr Val Tyr Lys Asn Gln Gln Ala Glu Leu Leu Leu Leu Ile Asp   251

ATC TCT TCA AGG AAA AAA TTC CAA ACC ATC CTG CAA AAA AGC CGT GAA CGA TAT CAG CTG CTG ATT CAA AAT TCC ATT GAT ACC ATT GCG  1036
Ile Ser Ser Arg Lys Lys Phe Gln Thr Ile Leu Gln Lys Ser Arg Glu Arg Tyr Gln Leu Leu Ile Gln Asn Ser Ile Asp Thr Ile Ala   281

GTG ATT CAC AAT GGA AAA TGG GTA TTT ATG AAT GAA TCG GGA ATT TCC CTG TTT GAA GCG GCT ACA TAT GAG GAT TTA ATT GGC AAA AAC  1126
Val Ile His Asn Gly Lys Trp Val Phe Met Asn Glu Ser Gly Ile Ser Leu Phe Glu Ala Ala Thr Tyr Glu Asp Leu Ile Gly Lys Asn   311

ATA TAC GAT CAG CTG CAT CCT TGC GAT CAC GAG GAT GTA AAA GAG AGA ATC CAA AAC ATT GCC GAG CAA AAA ACA GAA TCT GAA ATT GTC  1216
Ile Tyr Asp Gln Leu His Pro Cys Asp His Glu Asp Val Lys Glu Arg Ile Gln Asn Ile Ala Glu Gln Lys Thr Glu Ser Glu Ile Val   341

AAG CAA TCC TGG TTC ACC TTT CAG AAC AGG GTC ATC TAT ACG GAG ATG GTC TGC ATT CCG ACG ACC TTT TTT GGT GAA GCG GCC GTC CAG  1306
Lys Gln Ser Trp Phe Thr Phe Gln Asn Arg Val Ile Tyr Thr Glu Met Val Cys Ile Pro Thr Thr Phe Phe Gly Glu Ala Ala Val Gln   371

GTC ATT CTT CGG GAC ATC TCA GAG AGA AAA CAA ACA GAA GAA TTG ATG CTG AAA TCG GAA AAA TTA TCA ATC GCA GGG CAG CTC GCG GCG  1396
Val Ile Leu Arg Asp Ile Ser Glu Arg Lys Gln Thr Glu Glu Leu Met Leu Lys Ser Glu Lys Leu Ser Ile Ala Gly Gln Leu Ala Ala   401

GGA ATC GCC CAT GAG ATC CGC AAC CCT CTT ACA GCG ATC AAA GGA TTT TTA CAG CTG ATG AAA CCG ACA ATG GAA GGC AAC GAA CAT TAC  1486
Gly Ile Ala His Glu Ile Arg Asn Pro Leu Thr Ala Ile Lys Gly Phe Leu Gln Leu Met Lys Pro Thr Met Glu Gly Asn Glu His Tyr   431

TTT GAT ATT GTG TTT TCT GAA CTC AGC CGT ATC GAA TTA ATA CTC AGT GAA CTG CTC ATG CTG GCG AAA CCT CAG CAA AAT GCT GTC AAA  1576
Phe Asp Ile Val Phe Ser Glu Leu Ser Arg Ile Glu Leu Ile Leu Ser Glu Leu Leu Met Leu Ala Lys Pro Gln Gln Asn Ala Val Lys   461

GAA TAT TTG AAC TTG AAA AAA TTA ATT GGT GAG GTT TCA GCC CTG TTA GAA ACG CAG GCG AAT TTA AAT GGC ATT TTT ATC AGA ACA AGT  1666
Glu Tyr Leu Asn Leu Lys Lys Leu Ile Gly Glu Val Ser Ala Leu Leu Glu Thr Gln Ala Asn Leu Asn Gly Ile Phe Ile Arg Thr Ser   491

TAT GAA AAA GAC AGC ATT TAT ATA AAC GGG GAT CAA AAC CAA TTA AAG CAG GTA TTC ATT AAT TTA ATC AAA AAT GCA GTT GAA TCA ATG  1756
Tyr Glu Lys Asp Ser Ile Tyr Ile Asn Gly Asp Gln Asn Gln Leu Lys Gln Val Phe Ile Asn Leu Ile Lys Asn Ala Val Glu Ser Met   521

CCT GAT GGG GGA ACA GTA GAC ATT ATC ATA ACC GAA GAT GAG CAT TCT GTT CAT GTT ACT GTC AAA GAC GAA GGG GAA GGT ATA CCT GAA  1846
Pro Asp Gly Gly Thr Val Asp Ile Ile Ile Thr Glu Asp Glu His Ser Val His Val Thr Val Lys Asp Glu Gly Glu Gly Ile Pro Glu   551

AAG GTA CTA AAC CGG ATT GGA GAG CCA TTT TTA ACA ACA AAA GAA AAA GGT ACG GGG CTT GGA TTA ATG GTG ACA TTT AAT ATC ATT GAA  1936
Lys Val Leu Asn Arg Ile Gly Glu Pro Phe Leu Thr Thr Lys Glu Lys Gly Thr Gly Leu Gly Leu Met Val Thr Phe Asn Ile Ile Glu   581

AAC CAT CAG GGA GTT ATA CAT GTG GAC AGC CAT CCT GAA AAA GGC ACA GCG TTT AAA ATT TCA TTT CCA AAA AAA TAA AAACAACGGCTTAAA  2029
Asn His Gln Gly Val Ile His Val Asp Ser His Pro Glu Lys Gly Thr Ala Phe Lys Ile Ser Phe Pro Lys Lys                       606

CGCCGTTGTTTATCGTCTGCATTGCTTCACGTTTTTTTAATACAAATAATTCTAAACGGTCTAGGCCTTCTCTCAGCGTGTCCATTGAGCATGC                          2123
```

FIG. 3. Nucleotide sequence of the *spoIIJ* gene. The sequence of the *Sac*I-*Sph*I fragment is shown with the predicted sequence of the SpoIIJ protein. Putative transcription termination signals are indicated by inverted arrows, the translation termination codon of the upstream gene is boxed, and the ribosome-binding site of *spoIIJ* is underlined twice. The 5 bp duplicated by the Tn*917*ΩHU19 insertion are overlined. Bold underlines point to the regions highly conserved in the transmitter domain of the sensor proteins (34). This sequence has been deposited in the GenBank data library under accession number M29450.

unfruitful, suggesting that its product is essential for cell viability. In similar experiments with a 1.3-kb *Sph*I-*Pvu*II fragment, small transformants were obtained which could grow on DS agar plates as "patches" but not as isolated colonies and which grew slowly in DS liquid medium. It seems likely that an intact gene has been reconstituted after integration of the plasmid in these transformants but is poorly expressed due to the absence of the promoter on the cloned DNA fragment. The predicted sequence of the product of this open reading frame was compared with the sequences of known proteins contained in the GenBank and EMBL data bases. A strong similarity was found with tyrosine aminotransferase from rat; some less extensive similarities were also observed with the *E. coli tyrB* and *hisC* products, with the *B. subtilis hisH* product, and with some aspartate aminotransferases of mitochondrial and mamma-

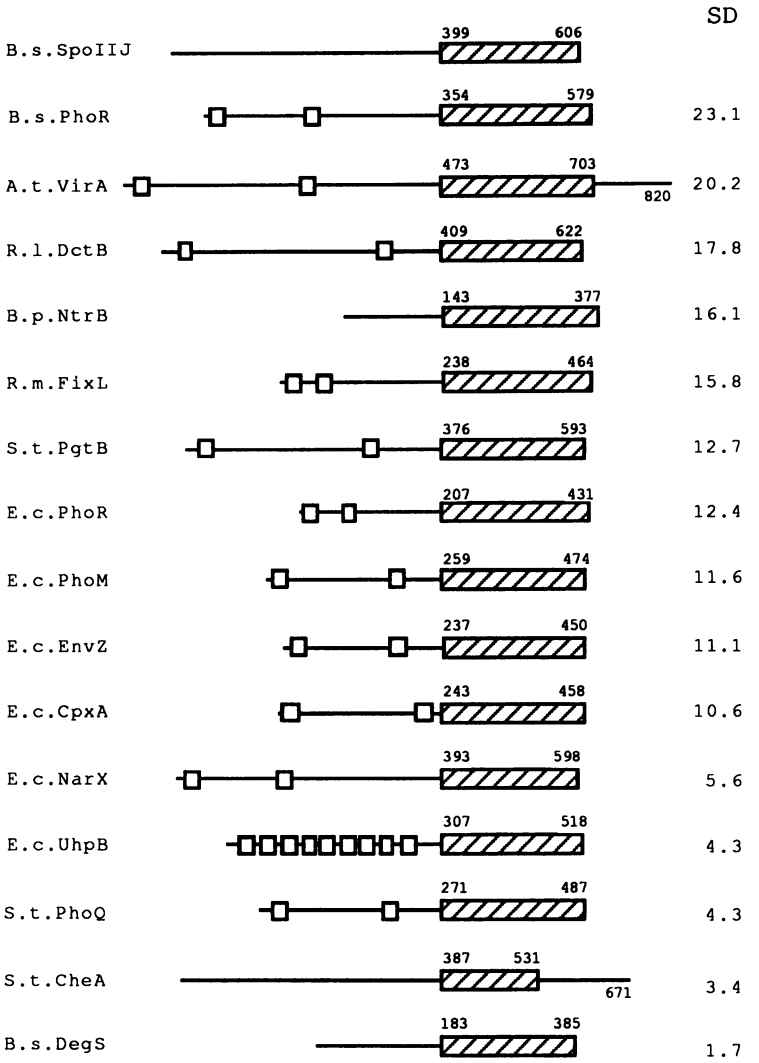


FIG. 4. Summary of similarities of the *spoIIJ* product with members of the sensor class of environmentally responsive genes. Hatched boxes represent the transmitter domains aligned from their amino termini. Empty boxes indicate transmembrane domains (mostly putative). Numbers above and under the sequences indicate amino acid residues. The ALIGN program (6) was used to calculate the relatedness of two sequences by determining the number of standard deviations (SD value) separating the maximum alignment from the average of 20 randomized sequences. In these comparisons the mutation data matrix (6) was used and a penalty of 10 was assigned to introduced gaps. Detailed alignments and references for most of the sensor sequences can be found in references 21, 26, and 34. Other sequences are found in references 10, 20, and 31. Abbreviations: B.s., *B. subtilis*; A.t., *Agrobacterium tumefaciens*; R.l., *Rhizobium leguminosarum*; B.p., *B. parasponiae*; R.m., *Rhizobium meliloti*; S.t., *Salmonella typhimurium*; E.c., *E. coli*.

lian origin (data not shown). Together, these sequence similarities suggest that the product of this open reading frame could be some essential unidentified aminotransferase, and we propose to name this gene *uat*. Since the absolute requirement for this gene was observed in rich media, *uat* cannot encode an enzyme involved in an usual amino acid-biosynthetic pathway. We tried to identify its end product by adding various compounds to the DS agar plates (such as diaminopimelate or pyridoxine) in order to correct the growth defect of the *uat* leaky mutants described above. These attempts were unsuccessful.

**A transmitter domain in the SpoIIJ protein.** The open reading frame interrupted by the HU19 insertion encodes a 69,127-molecular-weight polypeptide without any remarkable feature in its composition. It contains 87 acidic residues and 88 basic ones (including histidine). There are no large hydrophobic clusters, and the hydropathy plot indicates that the SpoIIJ protein is apparently not associated with the membrane (not shown). However, comparison with the protein sequences deposited in the data bases revealed the presence in the carboxy-terminal part of SpoIIJ of several motifs (underlined in Fig. 3) that are conserved in various procaryotic proteins, such as NtrB from *Klebsiella pneumoniae* and *Bradyrhizobium parasponiae* and PhoM and CpxA from *E. coli*. These proteins are members of the "sensor" class of the two-component regulatory systems, a family of proteins which transduce environmental signals to a cognate member of a second class of regulatory proteins, which in turn induces an adaptative response, usually by activating transcription of specific gene sets (27).

These sensor proteins share a conserved carboxy-terminal domain of about 210 residues, the "transmitter module," which is supposed to interact in pairwise fashion with a conserved acidic pocket found in the amino-terminal part of

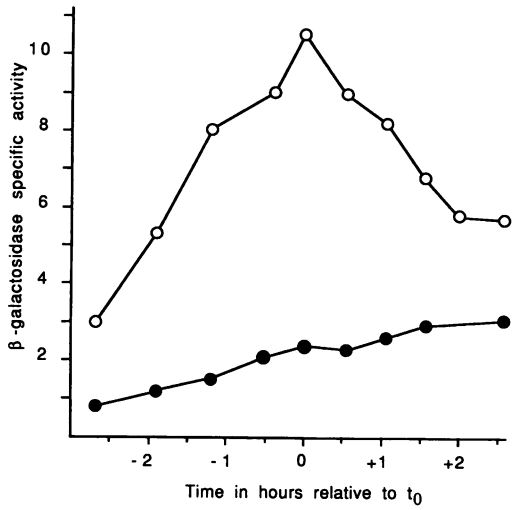


FIG. 5. Time course of *spoIIJ-lacZ* induction. Shown is the specific activity of β-galactosidase in strain JH642 (*spo0H*$^+$) (○) and in strain IS233 (*spo0H* Δ*Hin*dIII) (●) carrying a *spoIIJ-lacZ* fusion at the *amy* locus. The cells were grown in DS medium.

the effector proteins (17, 27). Most of these sensors are membrane bound and receive information from the external medium through a periplasmic domain. This is clearly not the case for SpoIIJ, which appears from its sequence to be a cytoplasmic protein. A thorough statistical comparison was made between the postulated transmitter domain of SpoIIJ and the corresponding region of other available sensorlike sequences (Fig. 4). Among 15 sequences tested (we took into account only one NtrB sequence), the closest relatedness was found with the *B. subtilis* PhoR protein, and the significance of the similarity with SpoIIJ was extremely high for nine others. In all cases the similarity did not extend outside of the transmitter module.

**Expression of the *spoIIJ* gene.** Transcription of *spoIIJ* could be predicted to start in the *Sac*I-*Nsi*I fragment, which overlaps the end of the upstream gene (including its putative transcription termination signal), and the beginning of the *spoIIJ* coding sequence (Fig. 2). This fragment was cloned upstream of a promoterless *lacZ* gene, and the fusion was introduced by a double recombination event into the chromosome of strain JH642 at the *amy* locus. Expression of *spoIIJ* was then followed by measuring β-galactosidase synthesis during growth and sporulation in DS medium (Fig. 5). Expression of *spoIIJ* was very weak compared with expression in other fusions and its timing was quite different from what has been observed with other stage II genes (cf. Fig. 1); *spoIIJ*-driven β-galactosidase synthesis increased continuously during vegetative growth and declined after the onset of sporulation. As a preliminary study of its dependence pattern, *spoIIJ* expression was studied in a *spo0H* mutant and was found to be severely decreased, the residual activity being barely higher than the background level measured in the absence of any fragment cloned upstream of *lacZ* (Fig. 5). On the contrary, the *spoIIJ* transposon mutation did not affect *spoIIJ* expression (data not shown). It was then possible to introduce a *lacZ* fusion at the *spoIIJ* locus (by recombining plasmid pTV55 into the resident Tn*917* transposon of strain MO845) and to measure its expression. No significant difference was found with the fusion at the *amy* locus (data not shown), indicating that all the *cis*-acting sequences required for *spoIIJ* transcription were carried by the *Sac*I-*Nsi*I fragment used in these experiments.

## DISCUSSION

The *spoIIJ* gene, previously defined by a single Tn*917* insertion, has now been cloned and characterized. It consists of a single cistron which is located 8.6 kb downstream of the *pstI* gene and is transcribed in the same direction as the *pts* operon. It is principally expressed during the exponential phase of growth, and its product is required for efficient transcription of the *spoIIA*, *spoIIE*, and *spoIIG* operons. The phenotype of a *spoIIJ* mutation fits with this latter result; although difficult to characterize due to its leakiness, a *spoIIJ* mutation leads to some stage 0 blockage and to the presence of aberrant stage II forms (A. Ryter, personal communication), similar results being obtained with the Tn*917*ΩHU19 insertion or with mutations induced by integrative plasmids carrying various internal fragments of *spoIIJ* (data not shown). The *spoIIJ* promoter has not been precisely identified but could be recognized by $\sigma^H$, since *spoIIJ* transcription depends on the presence of a wild-type *spo0H* gene. A possible $\sigma^H$-controlled promoter is found starting at position 103 in Fig. 3, GAAGGA-(18 bp)-GAATC. As a precedent, it has recently been shown that the P2 promoter of the *citG* gene is recognized by $\sigma^H$ during vegetative growth (25). A similar dependency for *spoIIJ* could partly explain the observed requirement for $\sigma^H$ in expression of the *spoIIE* and *spoIIG* operons (12, 18) that seem to be actually transcribed by $\sigma^A$-associated RNA polymerase (T. Kenney, K. York, P. Youngman, and C. Moran, Proc. Natl. Acad. Sci. USA, in press).

The nucleotide sequence of the *spoIIJ* gene has revealed that its product contains a canonical transmitter domain. Thus, *spoIIJ* belongs to a class of genes involved in sensing environmental stimuli and relaying this information by activating a cognate protein. Since SpoIIJ does not contain any membrane-associated domain, it is likely to be sensitive to the cytoplasmic level of some metabolite, which itself varies as the growth medium becomes exhausted. Expression of *spoIIJ* during vegetative growth correlates well with a role of its product in detecting starvation signals as soon as they occur.

Usually the two protein partners involved in signal transduction systems in bacteria are encoded by adjacent genes (27). Our sequence data as well as the phenotypes of in vitro-engineered mutations in the adjacent open reading frames rule out this possibility for *spoIIJ*. The obvious candidates are then the Spo0A and/or the Spo0F proteins, which contain a "receiver" module and are likely to be activated by some transmitter-containing protein (9, 36). Some unpublished data suggest that this could actually be the case. The *spo0A coi-1* mutation, which was selected by its catabolite-resistant sporulation phenotype, suppresses the Tn*917*ΩHU19 *spoIIJ* mutation (G. Olmedo and P. Youngman, personal communication). The sporulation-defective phenotype observed in the presence of multiple copies of the *spo0F* gene is corrected by overproducing the SpoIIJ protein (I. Smith, personal communication). A definite proof of the interaction between the *spoIIJ* product and Spo0A and/or Spo0F will be provided by the isolation of allele-specific suppressor mutations in these genes or by direct in vitro biochemical evidence.

All mutations that completely inactivate the *spoIIJ* gene give an oligosporogenous phenotype with production of 10 to 30% of the wild-type level of heat-resistant spores (data not shown). This strongly suggests the existence of other sensor proteins which can somehow replace SpoIIJ: either another protein is able to "crosstalk," albeit inefficiently, with the

SpoIIJ partner in a *spoIIJ* mutant, or multiple sensor proteins (among them SpoIIJ) are transmitting information to the same partner and switch its conformation to an active form. In this latter model, sporulation would be triggered once the concentration of active effector (Spo0A or Spo0F, for instance) reached a critical threshold. The absence of one sensor protein would then lead to only a modest deficiency in sporulation. It has been shown in three different systems that sensor proteins are kinases which autophosphorylate and can transfer their phosphate to their cognate effector (14, 15, 39). It seems likely that this is also the case for the *spoIIJ* product, but that remains to be demonstrated.

## ACKNOWLEDGMENTS

We are grateful to C. Karmazyn-Campelli for her assistance in some of the experiments reported here and to G. Gonzy-Tréboul for providing plasmids and unpublished information. We enjoyed stimulating discussions with T. Leighton, I. Smith, and A. Grossman.

This work was supported by a grant from the Centre National de la Recherche Scientifique (U.A. 136).

## LITERATURE CITED

1. **Ahmed, A.** 1985. A rapid procedure for DNA sequencing using transposon-mediated deletions in *Escherichia coli*. Gene **39:** 305–310.
2. **Anagnostopoulos, C., and J. Spizizen.** 1961. Requirements for transformation in *Bacillus subtilis*. J. Bacteriol. **81:**74–76.
3. **Biggin, M. D., T. J. Gibson, and G. F. Hong.** 1983. Buffer gradient gels and $^{35}S$ label as an aid to rapid DNA sequence determination. Proc. Natl. Acad. Sci. USA **80:**3963–3965.
4. **Bouvier, J., P. Stragier, C. Bonamy, and J. Szulmajster.** 1984. Nucleotide sequence of the *spo0B* gene of *Bacillus subtilis* and regulation of its expression. Proc. Natl. Acad. Sci. USA **81:** 7012–7016.
5. **Chak, K. F., H. DeLencastre, H. M. Liu, and P. J. Piggot.** 1982. Facile in vivo transfer of mutations between the *Bacillus subtilis* chromosome and a plasmid harbouring homologous DNA. J. Gen. Microbiol. **128:**2813–2816.
6. **Dayhoff, M. O., W. C. Barker, and L. T. Hunt.** 1983. Establishing homologies in protein sequences. Methods Enzymol. **91:** 524–545.
7. **Errington, J., and J. Mandelstam.** 1986. Use of *lacZ* gene fusions to determine the dependence pattern of the sporulation operon *spoIIA* in *spo* mutants of *Bacillus subtilis*. J. Gen. Microbiol. **132:**2967–2976.
8. **Ferrari, F. A., A. Nguyen, D. Lang, and J. A. Hoch.** 1983. Construction and properties of an integrative plasmid for *Bacillus subtilis*. J. Bacteriol. **154:**1513–1515.
9. **Ferrari, F. A., K. Trach, D. LeCoq, J. Spence, E. Ferrari, and J. A. Hoch.** 1985. Characterization of the *spo0A* locus and its deduced product. Proc. Natl. Acad. Sci. USA **82:**2647–2651.
10. **Friedrich, M. J., and R. J. Kadner.** 1987. Nucleotide sequence of the *uhp* region of *Escherichia coli*. J. Bateriol. **169:**3556–3563.
11. **Gonzy-Tréboul, G., M. Zagorec, M. C. Rain-Guion, and M. Steinmetz.** 1989. Phosphoenolpyruvate: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.
12. **Guzman, P., J. Westpheling, and P. Youngman.** 1988. Characterization of the promoter region of the *Bacillus subtilis spoIIE* operon. J. Bacteriol. **170:**1598–1609.
13. **Heusterspreute, M., V. Ha Thi, S. Emery, S. Tournis-Gamble, N. Kennedy, and J. Davison.** 1985. Vectors with restriction banks. IV. pJRD184, a 3793-bp plasmid vector having 43 unique cloning sites. Gene **39:**299–304.
14. **Igo, M. M., and T. J. Silhavy.** 1988. EnvZ, a transmembrane environmental sensor of *Escherichia coli* K-12, is phosphorylated in vitro. J. Bacteriol. **170:**5971–5973.
15. **Keener, J., and S. Kustu.** 1988. Protein kinase and phosphoprotein phosphatase activities of nitrogen regulatory proteins NTRB and NTRC of enteric bacteria: roles of the conserved amino-terminal domain of NTRC. Proc. Natl. Acad. Sci. USA **85:**4976–4980.
16. **Kenney, T. J., and C. P. Moran, Jr.** 1987. Organization and regulation of an operon that encodes a sporulation-essential sigma factor in *Bacillus subtilis*. J. Bacteriol. **169:**3329–3339.
17. **Kofoid, E. C., and J. S. Parkinson.** 1988. Transmitter and receiver modules in bacterial signaling proteins. Proc. Natl. Acad. Sci. USA **85:**4981–4985.
18. **LaBell, T. L., J. E. Trempy, and W. G. Haldenwang.** 1987. Sporulation-specific σ factor $\sigma^{29}$ of *Bacillus subtilis* is synthesized from a precursor protein, $P^{31}$. Proc. Natl. Acad. Sci. USA **84:**1784–1788.
19. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Miller, S. I., A. M. Kukral, and J. J. Mekalanos.** 1989. A two-component regulatory system (*phoP phoQ*) controls *Salmonella typhimurium* virulence. Proc. Natl. Acad. Sci. USA **86:** 5054–5058.
21. **Nohno, T., S. Noji, S. Taniguchi, and T. Saito.** 1989. The *narX* and *narL* genes encoding the nitrate-sensing regulators of *Escherichia coli* are homologous to a family of prokaryotic two-component regulatory genes. Nucleic Acids Res. **17:**2947–2957.
22. **Peng, Z. G., and R. Wu.** 1986. A simple and rapid nucleotide sequencing strategy and its application in analyzing a rice histone 3 gene. Gene **45:**247–252.
23. **Piggot, P. J., and J. G. Coote.** 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. **40:**908–962.
24. **Piggot, P. J., C. A. M. Curtis, and H. DeLencastre.** 1984. Use of integrational plasmid vectors to demonstrate the polycistronic nature of a transcriptional unit (*spoIIA*) required for sporulation of *Bacillus subtilis*. J. Gen. Microbiol. **130:**2123–2136.
25. **Price, V. A., A. M. Feavers, and A. Moir.** 1989. Role of $\sigma^H$ in expression of the fumarase gene (*citG*) in vegetative cells of *Bacillus subtilis* 168. J. Bacteriol. **171:**5933–5939.
26. **Ronson, C. W., P. M. Astwood, B. T. Nixon, and F. M. Ausubel.** 1987. Deduced products of C4-dicarboxylate transport regulatory genes of *Rhizobium leguminosarum* are homologous to nitrogen regulatory gene products. Nucleic Acids Res. **15:** 7921–7934.
27. **Ronson, C. W., B. T. Nixon, and F. M. Ausubel.** 1987. Conserved domains in bacterial regulatory proteins that respond to environmental stimuli. Cell **49:**579–581.
28. **Sandman, K., R. Losick, and P. Youngman.** 1987. Genetic analysis of *Bacillus subtilis spo* mutations generated by Tn*917*-mediated insertional mutagenesis. Genetics **117:**603–617.
29. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
30. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolite repression of bacterial sporulation. Proc. Natl. Acad. Sci. USA **54:**704–711.
31. **Seki, T., H. Yoshikawa, H. Takahashi, and H. Saito.** 1988. Nucleotide sequence of the *Bacillus subtilis phoR* gene. J. Bacteriol. **170:**5935–5938.
32. **Shimotsu, H., and D. Henner.** 1986. Construction of a single copy integration vector and its use in analysis of the *trp* operon of *Bacillus subtilis*. Gene **43:**85–94.
33. **Short, J. M., J. M. Fernandez, J. A. Sorge, and W. D. Huse.** 1988. λZAP: a bacteriophage λ expression vector with in vivo excision properties. Nucleic Acids Res. **16:**7583–7600.
34. **Stock, A., T. Chen, D. Welsh, and J. Stock.** 1988. CheA protein, a central regulator of bacterial chemotaxis, belongs to a family of proteins that control gene expression in response to changing environmental conditions. Proc. Natl. Acad. Sci. USA **85:** 1403–1407.
35. **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.
36. **Trach, K. A., J. W. Chapman, P. P. Piggot, and J. A. Hoch.** 1985. Deduced product of the stage 0 sporulation gene *spo0F* shares homology with the Spo0A, OmpR, and SfrA proteins. Proc. Natl. Acad. Sci. USA **82:**7260–7264.

37. **Trempy, J. E., J. Morrison-Plummer, and W. G. Haldenwang.** 1985. Synthesis of $\sigma^{29}$, an RNA polymerase specificity determinant, is a developmentally regulated event in *Bacillus subtilis*. J. Bacteriol. **161**:340–346.
38. **Weir, J., E. Dubnau, N. Ramakrishna, and I. Smith.** 1984. *Bacillus subtilis spo0H* gene. J. Bacteriol. **157**:405–412.
39. **Wylie, D., A. Stock, C. Y. Wong, and J. Stock.** 1988. Sensory transduction in bacterial chemotaxis involves phosphotransfer between Che proteins. Biochem. Biophys. Res. Commun. **151**: 891–896.
40. **Yamashita, S., H. Yoshikawa, F. Kawamura, H. Takahashi, T. Yamamoto, Y. Kobayashi, and H. Saito.** 1986. The effect of *spo0* mutations on the expression of *spo0A*- and *spo0F-lacZ* fusions. Mol. Gen. Genet. **205**:28–33.
41. **Youngman, P., J. B. Perkins, and R. Losick.** 1984. A novel method for the rapid cloning in *Escherichia coli* of *Bacillus subtilis* chromosomal DNA adjacent to Tn*917* insertions. Mol. Gen. Genet. **195**:424–433.
42. **Youngman, P., J. B. Perkins, and K. Sandman.** 1985. Use of Tn*917*-mediated transcriptional gene fusions to *lacZ* and *cat-86* for the identification and study of *spo* genes in *Bacillus subtilis*, p. 47–54. *In* J. A. Hoch, and P. Setlow (ed.), Molecular biology of microbial differentiation. American Society for Microbiology, Washington, D.C.