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Coupling of Flagellin Gene Transcription to Flagellar Assembly in *Bacillus subtilis*

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The regulation of flagellin gene expression in *Bacillus subtilis* was examined in vivo by means of a *lacZ* translational fusion to the flagellin structural gene (*hag*). We have tested the effects of two known mutations (*flaA4* and *flaA15*) in the major flagellar operon and of three deletions. One deletion was in frame in the *fliI* cistron, one was out of frame in the *fliK* cistron, and the last spanned about 21 kb of the *flaA* operon. In all instances, the expression of the flagellin gene was defective. Flagellin gene expression was restored in the strain with the 21-kb deletion by overexpression of the *sigD* gene under control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *spac* promoter. These results indicate that transcription of the flagellin gene is dependent on the formation of the flagellar basal body but that such a requirement can be bypassed by overexpression of *sigD*. Lack of expression of *hag* was observed in the presence of *flaD1*, *flaD2*, and *Δsin* mutations as well.

The bacterial flagellum is a dispensable complex organelle composed of the basal body, the hook, and the filament. Associated with the basal body are a number of proteins forming the motor and the switch. At least 50 gene products are involved in the structure and activity of *Salmonella* and *Escherichia coli* flagella (20, 21). An equivalent number has been described for the gram-positive bacterium *Bacillus subtilis* (28). A detailed analysis of the morphogenetic pathway of the *Salmonella* organelle indicates the sequential assembly of the various elements, from the inner components of the basal body to the last addition of the flagellin monomers to form the filament (17, 21). The order of the assembly process is, in general, a reflection of the hierarchy of expression of the genes that encode the assembled components (20). The *fliC* gene, encoding H1 flagellin, is transcribed only after the full assembly of the basal body has been completed. This regulation is mediated by the product of *flgM*. In the absence of a basal body, FlgM acts as an anti-sigma factor, inhibiting the action of σ^{28}, the sigma factor responsible for the transcription of *fliC* and other late genes, such as *fliDST*, *motAB*, etc. (8, 9, 27). Upon completion of the hook-basal body complex, FlgM is exported outside the cell by the flagellar export apparatus, relieving the inhibitory effect exerted on the transcription factor σ^{28} (16).

In *B. subtilis*, a single operon (*flaA*) extending over 23 kb codes for most of the flagellar structural and functional proteins (28). The operon is transcribed starting from a promoter recognized by the major form of RNA polymerase ($E\sigma^A$). Nothing is known about the regulation of its transcription. In addition to those in *flaA*, mutations in three other loci, i.e., *flaB*, *flaC*, and *flaD*, have been reported to produce a nonflagellate phenotype (31). It has been shown that the *flaB* gene is the structural gene for the RNA polymerase initiation factor σ^D and is located downstream of the *flaA* operon (14, 23, 28). The *sigD* (*flaB*) cistron can be transcribed independently of the *flaA* operon, and the encoded sigma factor is required for transcription of genes involved in motility, *motA* and *motB* (26); genes involved in production of autolysins, *lytBC* (18, 19); and the flagellin-encoding gene *hag* (25). These genes are unlinked to the *flaA* operon. Disruption of *sigD* leads to absence of motility, of flagellar filaments, and of transcription of *hag* (14).

Little is known about the *flaC* locus, whereas *flaD*, also known as *sin*, has been implicated directly in the transcription of *sigD* (*flaB*) when in the allelic form *flaD1* and more generally in flagellation and motility as a null mutant (7, 18, 22, 34).

Frameshift mutations in the *fliM*, *fliY*, *flhA*, and *flhF* cistrons of the *flaA* operon confer a Mot^- and Fla^- phenotype. In addition, transcription of the *hag* gene was impaired, as determined only qualitatively by the absence of blue colonies with mutant strains bearing a *hag-lacZ* fusion (2–4, 39). To test the hypothesis of feedback control of *hag* transcription upon the completion of the flagellar basal body in *B. subtilis*, we have measured the transcription of *hag* in five mutants of the *flaA* operon and also tested the effect of *flaD* mutations.

MATERIALS AND METHODS

Bacterial strains. All *B. subtilis* strains used in this study are listed in Table 1. The parent strain for the *hag-lacZ* translational fusion was CB123 (24), a derivative of JH642 (*trpC2 pheA1*), with plasmid pDM632 inserted at the *hag* locus. Plasmid pDM632 is based on pJH101 (6) and bears a flagellin–β-galactosidase fusion. DNAs from strain CB123 and plasmid pDM632 were kindly supplied by D. B. Mirel. *E. coli* DH5α *supE44 lacU169* (Φ80 *lacZ ΔM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* was used for transformation and plasmid propagation, and LB medium (10 g of tryptone [Difco], 5 g of yeast extract, 10 g of NaCl, and water to 1 liter [pH 7.1]) was used. Competent *E. coli* cells were prepared by the method of Hanahan (11). Transformation of competent *B. subtilis* cells was performed by the method of Hoch et al. (15). Selection for resistance to chloramphenicol (5 μg/ml) and kanamycin (2 μg/ml) was done on nutrient agar plates. Selection for bleomycin resistance (5 μg/ml) was done on Schaeffer sporulation medium (33). Motility was scored on swarm plates as described elsewhere (1).

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

Strain	Genotype[a]	Source or reference
CB123	*trpC2 pheA1 hag::lacZ cat*	24
PB168	*trpC2*	
PB1424	*hisB2 trpC2 metD4*	Laboratory stock
PB1764	*thyA1 thyB1 trpC2 ilvA1 pyrD*	QB943 (R. Dedonder)
PB2540	*metB5 ile-1 dnaE*	
PB5060	*purA16 fliG15 ilvA1*	L5256 (31)
PB5060/1	*hisB2 trpC2 fliG15*	PB5060 → PB1424[b]
PB5078	*hisB2 trpC2 metD4 ΔfliK*	This work[c]
PB5084	*purA16 metB5 ilvA1 flaD1*	L5417 (31)
PB5085	*purA16 metB5 ilvA1 flaD2*	L5329 (D. Karamata)
PB5088	*thyA1 thyB1 trpC2 ilvA1 flaA4*	L5395
PB5090	*leuA8 metB5 hisA1 Δsin::phl*	IS720 (22)
PB5114	*trpC2 ΔfliI*	This work[c]
PB5116	*hisB2 trpC2 metD4 hag::lacZ cat*	CB123 → PB1424[b]
PB5117	*hisB2 trpC2 metD4 Δsin::phl hag::lacZ cat*	PB5090 → PB5116[b]
PB5118	*metB5 ile-1 dnaE hag::lacZ cat*	CB123 → PB2540[b]
PB5119	*metB5 ile-1 flaD1 hag::lacZ cat*	PB5084 → PB5118[b]
PB5120	*metB5 ile-1 flaD2 hag::lacZ cat*	PB5085 → PB5118[b]
PB5123	*hisB2 trpC2 metD4 ΔfliK hag::lacZ cat*	CB123 → PB5078[b]
PB5125	*hisB2 trpC2 fliG15 hag::lacZ cat*	CB123 → PB5060/1[b]
PB5129	*trpC2 ΔflaA::kan*	This work[c]
PB5130	*trpC2 sigD*::pDBfla3 *kan*	This work[c]
PB5131	*trpC2 ΔflaA::kan hag::lacZ cat*	CB123 → PB5129[b]
PB5132	*trpC2 sigD*::pDBfla3 *kan hag::lacZ cat*	CB123 → PB5130[b]
PB5133	*trpC2 hag::lacZ cat*	CB123 → PB168[b]
PB5134	*thyA1 thyB1 trpC2 ilvA1 flaA4 hag::lacZ cat*	CB123 → PB5088[b]
PB5135	*thyA1 thyB1 trpC2 ilvA1 pyrD hag::lacZ cat*	CB123 → PB1764[b]
PB5136	*trpC2 ΔfliI hag::lacZ cat*	CB123 → PB5114[b]

[a] Δ*fliK* is an out-of-frame deletion of 100 bp in the *fliK* cistron; Δ*fliI* is an in-frame deletion of 329 bp in the *fliI* cistron; and Δ*flaA* is a deletion of about 21 kbp in the *flaA* operon. *cat*, *kan*, and *phl* are the determinants for resistances to chloramphenicol, kanamycin, and phleomycin, respectively.

[b] Constructed by transformation. The arrow points from donor to recipient.

[c] Construction of these strains is described in the text.

β-Galactosidase assays. Samples were taken during growth in Schaeffer sporulation medium (33).

All assays were performed on triplicate samples, and the values were averaged. β-Galactosidase specific activity is expressed in Miller units.

Construction of deletion mutant strains. A mutant with a deletion of 100 bp inside the *fliK* cistron was obtained, starting from plasmid pUF7-5, a derivative of pJH101 (6) carrying a *Pst*I fragment of 1,706 bp (from positions 4022 to 5728 of the published sequence [reference 1 and EMBL accession no. X56049]) (Fig. 1). The plasmid was digested with *Pvu*II and *Nsi*I, enzymes for which there is a unique site in the insert. The *Nsi*I site was made blunt by treatment with phage T4 DNA polymerase, and the plasmid was religated. The plasmid was used to transform competent PB1424 cells, with selection for chloramphenicol resistance. Transformants were screened by Southern hybridization for homogenotes bearing the deletion. One homogenote was grown overnight in the absence of the antibiotic, plated, and screened for Cm^s colonies. DNA from Cm^s segregants was checked by Southern hybridization. The strain obtained was named PB5078.

An in-frame deletion in the *fliI* cistron was obtained by several steps. First, a 170-bp *Cla*I-*Pst*I fragment was cloned in pJH101; this corresponds to an internal part of *fliI* (the *Cla*I site is at position 2959 and the *Pst*I site is at position 3129 of the sequence given in reference 1). Subsequently, in the *Hin*dIII and *Cla*I sites of the above-mentioned plasmid a 681-bp *Hin*dIII-*Cla*I fragment, derived from the 5′ end of *fliI* (*Hin*dIII at position 1950 and *Cla*I at position 2630), was inserted. The resulting plasmid had a deletion of 329 bp between the two *Cla*I sites. To set the deletion in frame, the plasmid was restricted with *Cla*I and the extensions were filled in with Klenow fragment and religated. As a consequence, a *Bgl*II site is eliminated and a new *Nru*I site is formed. The final integrative plasmid porf4 was used to transform strain PB168; homogenotes produced by gene conversion were screened and stable haploid deletion mutants were obtained as described above (strain PB5114).

A strain with a deletion of approximately 21 kb of the *flaA* operon was constructed by gene replacement, through a double crossover and insertion of a kanamycin resistance determinant. To this purpose a 1.8-kb fragment, corresponding to the downstream part of the *flaA* operon, from cistron *cheW* to *sigD* (28), was cloned in plasmid pJM114 (29). The fragment was obtained from a pGEM-4Z derivative, carrying a 10-kb *Bam*HI-*Eco*RI segment, cloned from λA21 (13). Following restriction with *Eco*RI, the ends were made blunt by treatment with Klenow fragment and then restricted with *Pst*I. The 1.8-kb fragment was purified and cloned into pJM114 digested with *Pst*I and *Sma*I. The resulting plasmid was called pDBfla3. The upstream fragment of 504 bp was from *Eco*RI (made blunt) to *Hpa*II, at positions 1 and 504, respectively, of the published sequence (1). The fragment was cloned in plasmid pDBfla3 cut with *Xho*I, made blunt, and digested with *Cla*I. The plasmid obtained, pDBflaK, was linearized with *Not*I and used to transform strain PB168, with selection for kanamycin resistance (2 μg/ml). One transformant with the desired deletion was used for subsequent analysis (PB5129).

All plasmid constructions were confirmed by restriction analysis and DNA sequence determination. All bacterial strains with deletions were analyzed by DNA restriction with various enzymes and by Southern blotting. DNA hybridization to suitable probes was detected by chemiluminescence (ECL; Amersham).

DNA sequence analysis. The *flaA15* mutant allele was cloned by PCR. Chromosomal DNA extracted from strain PB5060 was amplified by use of two synthetic oligonucleotides, a forward primer (18-mer; 5′ GATTTTGCAAAACTGCTG 3′) corresponding to the nucleotide sequence upstream of the *Hin*dIII site (position 585 of the sequence published in reference 1) and a backward primer (18-mer; 5′ GAAGACAAAC ATCCGCTT 3′) corresponding to the complementary strand, downstream of the *Bam*HI site (position 1241). The PCR was run for 35 cycles with the following parameters: denaturation, 3 min at 93°C for the first cycle and 1 min at 92°C thereafter; annealing, 1 min at 53°C; and elongation, 1 min at 72°C. The reagents and the thermocycler (Perkin-Elmer Cetus Corp.) were used in accordance with the manufacturer's instructions. The PCR product was purified from an agarose gel, digested with *Hin*dIII and *Bam*HI, and cloned in pBluescript (Stratagene). Two independent PCRs were performed, and two clones were sequenced by the chain termination method (32) using T7 DNA polymerase (Pharmacia LKB) and SP6 and T7 promoters or custom-made primers. Oligonucleotides were

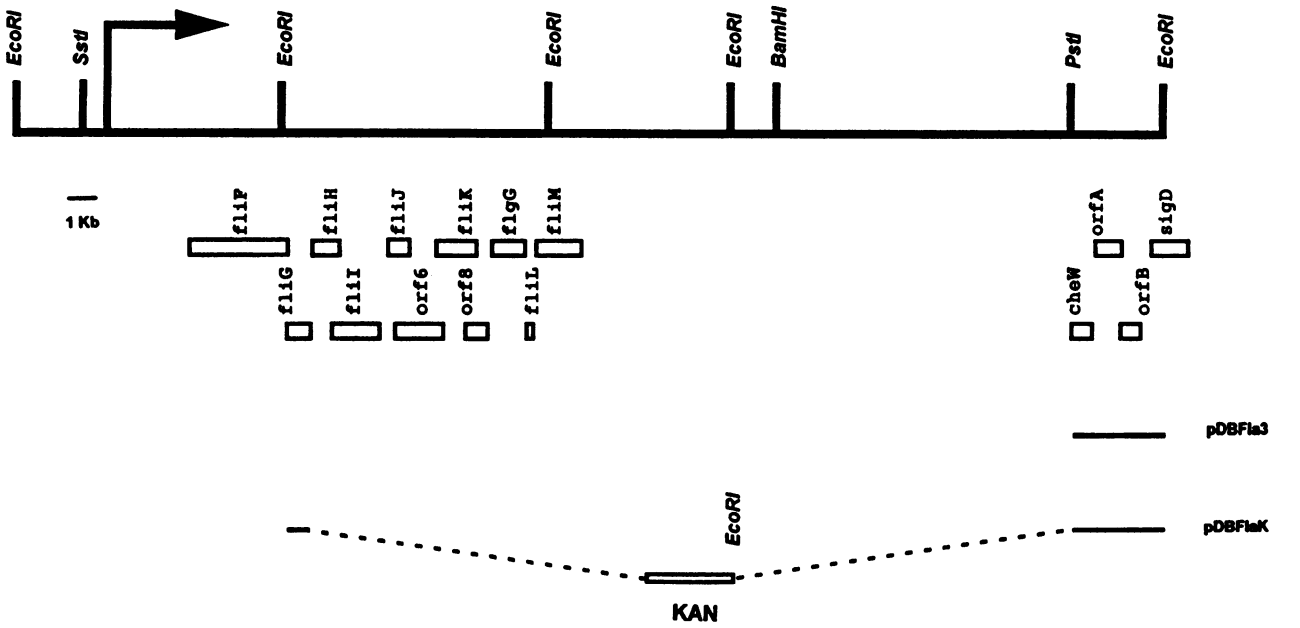

FIG. 1. Map of the *flaA* operon. The heavy line at the top represents the restriction map of the region, showing the five *Eco*RI sites and additional sites mentioned in the text. The arrow represents the major promoter. Only relevant open reading frames are shown. The positions and extensions of the fragments present in plasmids pDBfla3 and pDBflaK are shown at the bottom. KAN indicates the kanamycin resistance determinant of plasmid pJM114. Adapted from reference 27.

synthesized by the phosphoramidite method by means of a Cyclon Plus DNA synthesizer (Milligen Biosearch).

Plasmid construction. To obtain overproduction of σ^D, we cloned the *sigD* gene in a multicopy plasmid under control of the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible *spac* promoter (38). The coding sequence of *sigD* was obtained by PCR, performed with chromosomal DNA derived from strain PB168 (*trpC2*). The forward primer (SIG101) was a 31-mer with the sequence 5′ ATCCGTCGACAATGCAATCCTTG AATTATGA 3′; the underlined bases correspond to the beginning of *sigD* downstream of the ribosome binding site (14). The start codon is doubly underlined. The 5′ part of the primer was designed so as to contain a *Sal*I recognition site. The backward primer (SIG102) was a 35-mer derived from the sequence just downstream of the stop codon of *sigD* and with a *Sph*I recognition site at its 5′ end. The sequence of SIG102 was 5′ ACATGCATGCATGTCACTCGCTAACCATGAAA TTC 3′, with the sequence derived from *sigD* underlined (14). The PCR was run for 35 cycles with the following parameters: denaturation, 3 min at 93°C for the first cycle and 50 s at 92°C thereafter; annealing, 1 min at 50°C; and extension, 40 s at 72°C. The PCR product was purified by agarose gel, digested with *Sal*I and *Sph*I, and cloned into the replicative plasmid pDG148, in the polylinker region downstream of the *spac* promoter (35).

RESULTS AND DISCUSSION

Transcription of the *hag-lacZ* fusion in strains with the *flaA4*, *fla15*, and Δ*fliK* mutations. Strains with mutations originally designated *flaA4* and *flaA15*, isolated by Grant and Simon (10) and by D. Karamata (as described in reference 30) are autolysin deficient and flagellumless (12, 31); the *flaA15* mutation has been localized to the *fliG* cistron (previously called *orf2*; see below), whose deduced product is part of the motor switch (1, 13). The *flaA4* mutation could not be located precisely, but it is in a different cistron (13). The Δ*fliK* mutation is an out-of-frame deletion in *fliK* (previously *orf7*), whose product is similar to that of the *Salmonella typhimurium fliK* gene, reported to be responsible for control of the hook length (1). The expression of the *hag* gene, measured as β-galactosidase activity of a *hag-lacZ* fusion integrated at the *hag* locus, was abolished in the presence of *flaA15* and Δ*fliK* mutations and strongly reduced (8% residual activity) in strains with the *flaA4* mutation (Fig. 2A and Table 2). The residual expression observed for strain PB5088 (*flaA4*) is probably due to the leakiness of the *flaA4* mutation, as already reported (13). We conclude that the three mutations negatively affect the transcription of the *hag* gene.

Characterization of the *fliG15* mutation. To reach a better understanding of the mechanism through which an alteration of the switch may affect the expression of the flagellin gene, we determined the nature of the *fliG15* (originally designated *flaA15*) mutation. This was accomplished by PCR amplification of the chromosomal region corresponding to the *fliG* cistron from strain PB168, cloning, and sequencing. The *fliG15* mutation is a deletion of two bases (one G and one T at positions 632 and 633, respectively, of the published sequence) generating a nonsense codon at position 36 of the amino acid sequence. Hence the absence of expression of the *hag-lacZ* fusion in the presence of the *fliG15* and Δ*fliK* mutations could be due to a polar effect on downstream genes, in particular, *sigD*. Support for this interpretation could be found in the fact that strains with *fliG15* have a Lyt$^-$ phenotype (31), as do strains with the out-of-frame deletion Δ*fliK* (12).

An in-frame deletion of *fliI*. To test for such a possible polar effect, we introduced an in-frame deletion in the *fliI* cistron (previously designated *orf4*). The FliI protein is related to the catalytic subunits of F_0F_1 ATPase, with conservation of amino acid sequences (boxes A and B) thought to be involved in nucleotide binding and (box C) known to react with dicyclo-

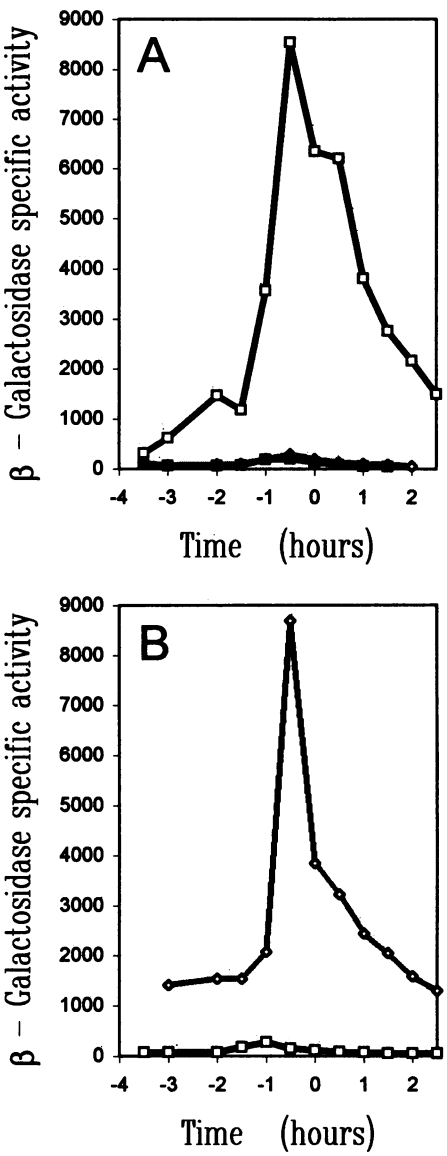

FIG. 2. Specific activities of β-galactosidase from a *hag-lacZ* fusion in wild-type and mutant strains. Cells were grown in Schaeffer sporulation medium, samples were removed at various times, and β-galactosidase specific activities in Miller units were determined. Symbols for panel A: □, PB5116 (wild type); ◇, PB5123 (Δ*fliK*); ■, PB5117 (Δ*sin*). Symbols for panel B: ◇, PB5133 (wild type); □, PB5136 (Δ*fliI*). On the time scale, zero indicates the transition from exponential growth to stationary phase.

hexylcarbodiimide (1, 36, 37). The function of FliI is not known, but it is suspected to be involved in the translocation of flagellar proteins (5). The coding sequence is 1,320 nucleotides long, and the deletion extends from nucleotide 2631 to nucleotide 2960. Four nucleotides were inserted to set the deletion in frame (see Materials and Methods). As a consequence of the deletion, boxes B and C of the deduced protein product are missing.

The deletion was constructed in vitro in an integrative plasmid, carrying a chloramphenicol resistance marker, selectable in *B. subtilis*. Substitution of the wild-type allele was obtained by integration of the plasmid carrying the deletion into the chromosome of strain PB168, with screening for homogenotes by Southern hybridization and for loss of the inserted plasmid by growth in the absence of selection for chloramphenicol resistance. The derived strain (PB5114) was Mot$^-$, and the expression of the *hag-lacZ* fusion was completely abolished (Fig. 2B and Table 2). Since the deletion is in frame, the effect of such a mutation cannot be attributed to lack of expression of any downstream gene, particularly that of *sigD*. Nevertheless, the mutation apparently affected the activity of sigma D factor, since the mutant strain had a Lyt$^-$ phenotype, not extreme but comparable to the one presented by strains with the *fliG15* mutation (Fig. 3B and D).

TABLE 2. β-Galactosidase activity expressed by a *hag-lacZ* fusion in *B. subtilis* mutants

Relevant mutation or plasmid	Activity (%) relative to that of parental strain
None (wild-type strain PB5116)	100
fliG15	0.7
Δ*fliK*	3.5
Δ*sin*	2.4
None (wild-type strain PB5135)	100
flaA4	8.8
None (wild-type strain PB5133)	100
Δ*fliI*	3.2
Δ*flaA*	1
pDBfla3	22
None (wild-type strain PB5118)	100
flaD1	0.06
flaD2	0.7

The fact that transcription of *hag* is blocked in the mutant affected solely in the *fliI* cistron indicates that expression of the flagellin gene requires the completion of the assembly of the flagellar basal body.

Deletion of most of the *flaA* operon. The requirement for the presence of the basal body for expression of the flagellin gene implies a regulatory coupling between morphogenesis and gene expression. A plausible interpretation is that one (or more) gene product(s), normally assembled in the basal body, acts by repressing the expression of *hag*. To test this possibility we deleted most of the *flaA* operon from the chromosome of *B. subtilis*: the extent of the deletion was 21 kbp, from *fliG* to *cheW* (Fig. 1) (27). To obtain the deletion, an upstream fragment of DNA corresponding to nucleotides 1 to 504 and a downstream segment (1.8 kb, from *Pst*I to *Eco*RI) were cloned in pJM114 at either side of a kanamycin resistance determinant (29). First the downstream fragment was cloned in pJM114; *B. subtilis* transformants obtained were Mot$^+$ and Hag$^+$ as determined by swarm plate assays and assaying for β-galactosidase activity driven by the *hag-lacZ* fusion (strain pDB*fla3* [Table 2]). This outcome was expected from previous results, indicating that the *sigD* gene is active in a strain carrying such an insertion (23, 40). Following the cloning of the upstream sequence, the plasmid (pDBflaK) was linearized with *Not*I and used to transform strain PB168. The chromosomal DNAs of 10 Kan^r transformants were analyzed by *Eco*RI digestion and Southern hybridization with a suitable probe. All transformants were identical, and all had the expected deletion. The deletion-bearing strains were Mot$^-$ and Lyt$^-$ (Fig. 3C). Following transformation with DNA from strain CB123, transcription of the *hag-lacZ* fusion was determined. As reported in Table 2 (strain Δ*flaA*), no transcription of *hag* in the mutant strain was observed.

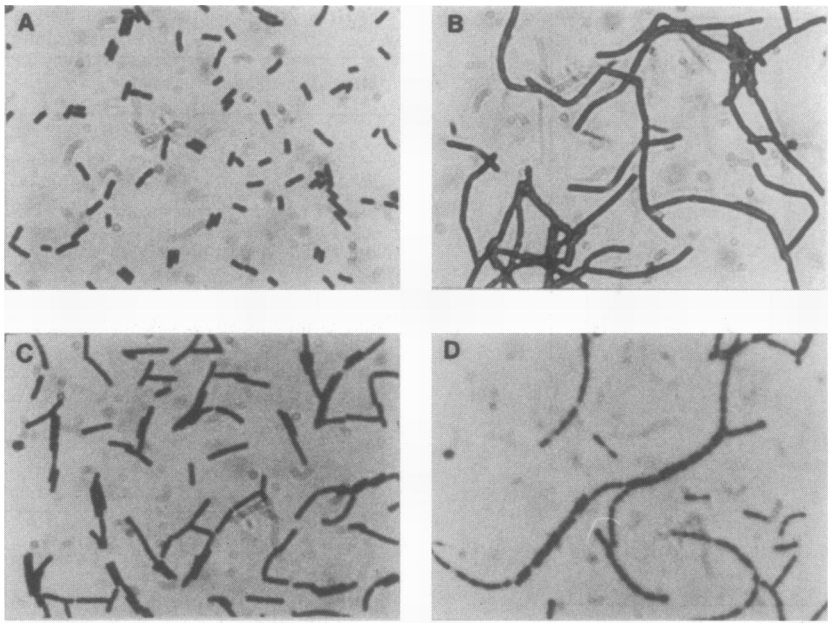

FIG. 3. Microscopic analysis of wild-type and mutant strains. (A) PB168 (wild type); (B) PB5114 (Δ*flaI*); (C) PB5129 (Δ*flaA*); (D) PB5060/1 (*fliG15*).

Overexpression of *sigD* restores transcription of the flagellin gene in the *flaA* deletion mutant. Ordal et al. have reported the presence in *B. subtilis* of a gene encoding a homolog of the enteric FlgM anti-sigma factor and mapping outside the *flaA* operon (28). The lack of expression of the flagellin gene in the deletion mutant could be explained by assuming that, in the absence of a functional hook-basal body complex, the FlgM product accumulates intracellularly and inactivates σ^D, thus reducing σ^D-dependent transcription of *hag* (16).

If the lack of expression of the flagellin gene in the Δ*flaA* mutant is due to the interaction of FlgM with the σ^D factor, it should be possible to overcome the negative effect of FlgM by overproducing the sigma factor. To obtain overproduction of σ^D, we cloned the *sigD* gene in a multicopy plasmid under control of the IPTG-inducible *spac* promoter (35, 38). The derived plasmid, pSigD, was introduced into strain PB5131, and β-galactosidase activity was monitored. The results are shown in Fig. 4. Following addition of the inducer, there is a net increase in β-galactosidase activity in the strain with the *sigD* gene under control of the *spac* promoter. The activity reaches a peak soon after addition of the inducer and then decreases. The highest activity observed was 969 Miller units, about 10-fold less activity than that usually obtained with Fla^+ strains (Fig. 2). This may be explained by the fact that the *flgM* gene is under control of a σ^D promoter (28); overexpression of *sigD* will increase transcription of *hag* but also that of *flgM*, thus reestablishing control over σ^D activity. Similar, and more extensive, results concerning the effect of the relative levels of FlgM and σ^{28} have been reported for *Salmonella* spp. (16). We can conclude that the presence of the flagellar basal body is not a requirement for *hag* transcription.

As reported above, the Δ*flaA* mutant is Lyt^-, suggesting that the level of autolysins is, at least partially, under control of σ^D and FlgM (14, 31). When strain PB5131(pSigD) was grown in the presence of IPTG, the cells were short rods, whereas the cells of the parental strain were filamentous (Fig. 5). Thus, as well as restoring expression of the flagellin gene, overproduction of σ^D corrects the Lyt^- phenotype.

Effect of Sin on *hag* transcription. We have also examined the effects of the *flaD1* and *flaD2* mutations and of a deletion of the *sin* gene upon the transcription of *hag*. In every mutant background, the level of expression of β-galactosidase driven by the *hag* promoter was very low (Fig. 2A and Table 2). The lack of expression of *hag* was independent of the growth stage and was observed at every time point analyzed. It should be

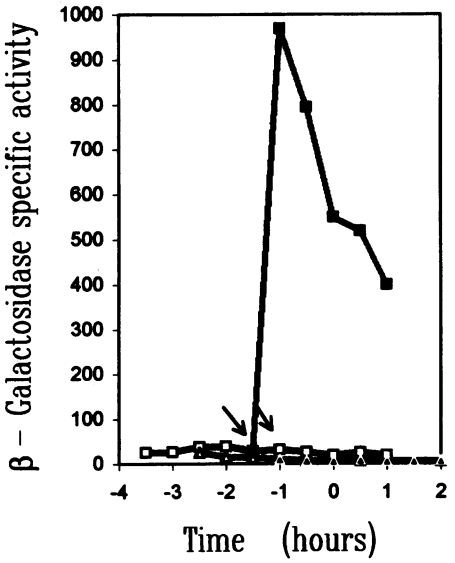

FIG. 4. Effect of induction of *sigD* on expression of a *hag-lacZ* fusion in a Δ*flaA* background. Cells were grown in Schaeffer sporulation medium, samples were removed at various times, and β-galactosidase specific activities in Miller units were determined. Symbols: squares, PB5131 (pSigD); triangles, PB5131; open symbols, without inducer; filled symbols, with IPTG. The arrows indicate the time of addition of IPTG (1 mM).

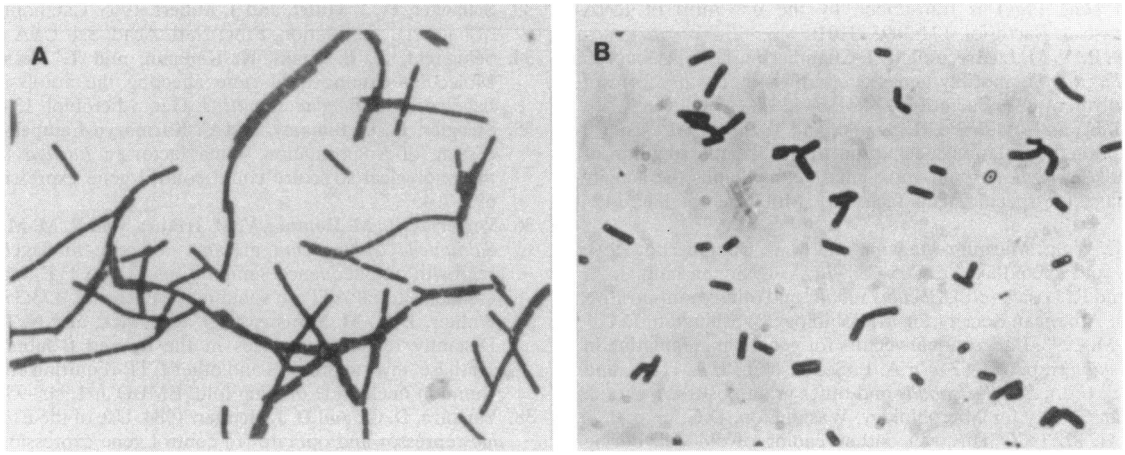

FIG. 5. Microscopic analysis of strain PB5131(pSigD) grown in the absence (A) or presence (B) of 1 mM IPTG.

noted, however, that Mirel et al. (26) detected a reduced but significant level of *hag* transcript in a *sin* deletion strain. Subtle differences in genetic background may explain this apparent discrepancy. According to Kuroda and Sekiguchi (18), the *flaD1* mutation abolishes transcription of *sigD*; this could explain the lack of transcription of *hag*. In addition, Lazarevic et al. (19) showed that the σ^D promoter of the *lytABC* operon is not active in a *flaD2* background. Null mutants of *sin* have an extreme Lyt$^-$ phenotype, with cells forming long chains (22). Sin could be acting as an activator of *sigD* transcription or as an activator of σ^D-dependent promoters. The two explanations do not necessarily exclude each other.

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