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Surface-Associated Flagellum Formation and Swarming Differentiation in *Bacillus subtilis* Are Controlled by the *ifm* Locus

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Knowledge of the highly regulated processes governing the production of flagella in *Bacillus subtilis* is the result of several observations obtained from growing this microorganism in liquid cultures. No information is available regarding the regulation of flagellar formation in *B. subtilis* in response to contact with a solid surface. One of the best-characterized responses of flagellated eubacteria to surfaces is swarming motility, a coordinate cell differentiation process that allows collective movement of bacteria over solid substrates. This study describes the swarming ability of a *B. subtilis* hypermotile mutant harboring a mutation in the *ifm* locus that has long been known to affect the degree of flagellation and motility in liquid media. On solid media, the mutant produces elongated and hyperflagellated cells displaying a 10-fold increase in extracellular flagellin. In contrast to the mutant, the parental strain, as well as other laboratory strains carrying a wild-type *ifm* locus, fails to activate a swarm response. Furthermore, it stops to produce flagella when transferred from liquid to solid medium. Evidence is provided that the absence of flagella is due to the lack of flagellin gene expression. However, restoration of flagellin synthesis in cells overexpressing σ^D or carrying a deletion of *flgM* does not recover the ability to assemble flagella. Thus, the *ifm* gene plays a determinantal role in the ability of *B. subtilis* to contact with solid surfaces.

Bacterial structures facing the cell surface play a critical role in establishing and maintaining interactions with the environment. In motile eubacteria, the flagellar organelle is the most complex and exposed extracytoplasmic structure and is made up of many proteins that are sequentially assembled from the cytoplasmic membrane outward. This highly regulated process is governed, at least in part, by the hierarchical expression of flagellar genes that are organized in classes in both the enteric bacteria and *Bacillus subtilis*. While class II genes encode structural and regulatory proteins needed for assembly of the hook-basal body, class III genes, whose expression depends on the late-flagellar-stage sigma factor (σ^{28} in the enterics and σ^D in *B. subtilis*), encode proteins required for the maturation of flagella and the chemosensory system (reviewed in references 1, 22, and 30).

The flagellum is essential for active movement of individual cells in a liquid environment (swimming) and for chemotaxis and plays an important role in interaction with surfaces as a sensor of medium viscosity (23) or as an adhesion tool (12). Flagellum-driven motility may help pathogens to reach their target, thus contributing to bacterial virulence (32).

Bacteria may experience two different life-styles, depending on whether they grow in liquid environments or are in contact with solid surfaces. The transition from free to sessile growth requires the sensing of yet unknown surface-related signals and their processing to trigger a productive interaction with the solid surface. This type of adaptation is particularly important for soil bacteria such as *B. subtilis* that rapidly shift from planktonic to sessile life, depending on microenvironmental conditions.

Among the best-characterized responses of flagellated bacteria to contact with solid surfaces is the cooperative behavior known as swarming motility. Swarming can be considered a strategy for rapid spread over solid surfaces in the environment and for active colonization of mucosal surfaces in infected hosts (2, 4). Swarming bacteria produce highly organized communities of elongated and aseptate cells that exhibit a remarkable increase in the number of flagella in comparison to short oligoflagellated cells growing in liquid media (8, 15, 17, 19, 39). Although it has been shown that swarming motility requires the integrity of the flagellar and chemotaxis systems in both gram-negative and gram-positive bacteria (5, 7, 11, 14, 17, 20, 31, 39, 41), almost nothing is known about the molecular mechanisms involved in the sensory transduction pathways of cell differentiation in response to solid clues.

Strikingly different colony patterns have been described for *B. subtilis*, depending on nutrient availability, agar concentration (36), and surfactin production (20). In this study, we describe the isolation of a *B. subtilis* hypermotile mutant that exhibits the ability to swim or swarm depending on whether it is propagated in liquid or on solid media. The hypermotile strain carries a mutation in the *ifm* locus described in 1969 by Grant and Simon (13) that affects the degree of motility and level of flagellation of *B. subtilis* during growth in liquid media. In contrast to the mutant, we found that the parental strain does not mount a swarming response and even stops to produce flagella when transferred from liquid to solid medium. Evidence is also provided that other motile *B. subtilis* strains

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

Strain	Genotype	Source, derivation, and/or reference
PB168	*trpC2*	168[a] (40)
PB25	*trpC2 hisB2*	J. Lederberg, SB25[a]
PB1424	*trpC2 hisB2 metD4*	Laboratory strain
PB1831	*trpC2 pheA1*	J. A. Hoch, JH642[a]
PB1927	*pheA1 sfp*	OKB105[a] (29)
PB5148	*trpC2 pheA1 flgMΔ80*	CB149[a] (26)
PB5249	*trpC2 pheA1 ifmP*	This study
PB5250	*trpC2 hagΔ717* (Kan[r])[d]	This study
PB5308	*trpC2 pheA1 pks*::P_{458} *hag-lacZ* (Cat[r])[d]	9
PB5309	*trpC2 pheA1 ifmP pks*::P_{458} *hag-lacZ* (Cat[r])[d]	PB5308 (tf) PB5249[b]
PB5332	*pheA1 ifmP sfp*	PB1927 (tf) PB5249[c]

[a] Previous designation of strain.
[b] Strain constructed by transformation, using donor DNA from PB5308.
[c] Strain constructed by transformation, using donor DNA from PB1927.
[d] Cat[r], chloramphenicol resistance.

carrying a wild-type *ifm* locus are unable to swarm and to produce flagella over solid surfaces.

MATERIALS AND METHODS

Bacterial strains and media. Table 1 lists the *B. subtilis* strains used in this study. For mapping purposes, a set of *B. subtilis* mutants constructed by several laboratories (38) was used. The strains were grown at 37°C in either tryptone-NaCl (1% tryptone, 0.5% NaCl [TrB]), nutrient broth, Schaeffer sporulation medium, or Luria-Bertani broth (LB). The media were routinely solidified with 1.5% agar unless otherwise noted. Surfactin production was assayed on blood agar plates (Columbia agar with sheep blood; Oxoid) as described by Nakano et al. (29). *Escherichia coli* DH5α *supE44 lacU169* (*ϕ80 lacΔZM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1* was used as the host for construction of recombinant plasmids. *E. coli* cells were grown in LB broth. When necessary, antibiotics were used at the following concentrations: 100 μg of ampicillin/ml; 1 μg of erythromycin/ml; 2.5 μg of kanamycin/ml; and 5 μg of chloramphenicol/ml.

Genetic techniques. *B. subtilis* strains were transformed with chromosomal or plasmid DNA by using the procedure of Kunst and Rapoport (21). Transduction mapping with the PBS1 phage was performed according to Hoch et al. (18). *E. coli* transformation was performed following standard protocols (37).

Motility assays. For swimming motility, strains were propagated in gelatin-agar (motility plate) having the following composition: 1% Bacto Peptone, 8% Bacto Gelatin, 1% Bacto Agar, 0.5% NaCl, and 25 μg of the appropriate growth requirement/ml (Table 1). Phenotypic assays for swarming were initiated by spotting 2 μl of an overnight culture at the center of tryptone-NaCl plates containing 1.5% agar (TrA). The plates were analyzed after growth for up to 24 to 48 h of incubation at 37°C. Bacteria were Gram stained for microscopy to evaluate the presence of elongated swarm cells (39). Flagellum staining was performed as described by Harshey and Matsuyama (16).

Construction of mutant strains. To construct strain PB5250 with a deletion of the flagellin-encoding gene (*hag*), a sequence of 717 bp was replaced with the kanamycin resistance determinant. First, a 330-bp DNA fragment corresponding to the region upstream of the *hag* coding sequence (from nucleotide 375 to nucleotide 45 with respect to the initiation of transcription) was amplified by PCR by using the primers HagB (5′-CG**GGATCC**CATTATTG TGAATCGCA AG-3′) and HagE (5′-CG**GAATTC**AAAAAAATCCTCACTTTTTTTGTGAG GAT-3′). The *Bam*HI (HabB) and *Eco*RI (HagE) recognition sequences are in bold. The template was chromosomal DNA of *B. subtilis* PB168. The amplified fragment was purified by acrylamide gel electrophoresis, electroeluted, and restricted with *Eco*RI and *Bam*HI. After purification, the DNA fragment was ligated into the *Eco*RI and *Bam*HI sites of plasmid pJM114 (33), thus generating pTCH1. The ligated plasmid was used to transform *E. coli* DH5α. A second DNA fragment of 180 bp, extending from codon 224 to codon 283 of the *hag* coding sequence, was obtained by PCR by using primers HagC (5′-CC**ATCGA T**CAACCAAGTTTCTTCTCAACGT-3′) and HagK (5′-GG**GGTACC**TGAGA AAGAATGTTGTTCTTTG-3′). The *Cla*I (HagC) and *Kpn*I (HagK) sites are in bold. The amplified DNA was restricted with *Cla*I and *Kpn*I, treated as above, and cloned into pTCH1, the pJM114 derivative digested with *Cla*I and *Kpn*I. The final plasmid, named pTCH2, contained two DNA fragments derived from PB168 flanking the kanamycin resistance determinant. The plasmid was verified by sequencing. The plasmid DNA (pTCH2) was linearized by *Bam*HI digestion and used to transform competent cells of PB168. After selection for kanamycin resistance, one transformant was characterized by PCR and named PB5250. To construct strain PB5332, we followed the marker congression procedure using DNA from the surfactin producer strain PB1927 as described by Nakano et al. (29). Selection was for Trp$^+$ transformants, followed by screening for surfactin production on blood agar plates.

β-Galactosidase activity assay. To measure β-galactosidase activity, overnight cultures in sporulation medium were diluted in fresh medium and samples (1.0 ml) were taken at 30-min intervals for reading of optical density at 525 nm (OD_{525}) and determination of β-galactosidase activity, which is expressed in modified Miller units (34).

RNA isolation and RT-PCR. Total RNA was purified from *B. subtilis* cultures grown in TrB or TrA for 6 h. Cells were harvested from plates by washing the surface of agar plates with cold diethylpyrocarbonate-treated water or collected from liquid cultures by centrifugation at 4,000 × *g* for 15 min. After being washed with diethylpyrocarbonate-treated water, about 1×10^8 bacterial cells were resuspended in 450 μl of RLT buffer (RNeasy Mini kit; QIAGEN) containing 0.35 g of glass beads (diameter 0.1 mm) and vortexed for 15 min to break the cells. Samples were centrifuged for 2 min at 10,000 × *g*, and the aqueous phase was removed. Two hundred microliters of absolute ethanol was added, and the mixture was applied to an RNeasy Mini Spin column (QIAGEN). After being digested with 40 Kunitz units of RNase-free DNase (QIAGEN) for 20 h, total RNA was eluted from the column according to the instructions of the manufacturer. An aliquot of the RNA was examined on agarose gel to ensure its integrity. Reverse transcription (RT)-PCRs were performed in one-step reactions. Up to 1 μg of RNA was mixed with 0.8 μM (each) primer in AMV/Tfl buffer (50 mM Tris HCl [pH 8.3], 50 mM KCl, 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM spermidine) containing 1.0 mM MgSO4, 0.1 mM deoxynucleoside triphosphate, 25 U Tfl polymerase (Promega), and 3.75 U of AMV reverse transcriptase (Promega) in a final volume of 25 μl. Reactions were incubated at 48°C for 60 min. PCR amplification was as follows: 30 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min for the *hag* gene; 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for *fliM*, *flgM*, *sigD*, and *cheW*. The following primers were used: HAGU1 (5′-CACAATATTGCAGCGCTTAA-3′) and HAGL1 (5′-TAATAATTGAAGTACGTTTTG-3′) for *hag*, BSFLIMU (5′-CTCCCAAAAT GAAATAGATG-3′) and BSFLIML (5′-TTCTCCATCTTGTTCACCTCT-3′) for *fliM*, FLGMF1 (5′-AATCAATTTGGAACACA-3′) and FLGMR3 (5′-ATT TGCGTCTACTTTGTA-3′) for *flgM*, BSSIGDU (5′-GAATTATGAAGATCA GGTG-3′) and BSSIGDL (5′-TTGTATCACTTTTTCCAGCAG-3′) for *sigD*, and CHEWF (5′-GGTAAATGGCAAAGAATATG-3′) and CHEWR (5′-AG CTTGATCGGGCACAG-3′) for *cheW*. Contamination by DNA was checked by performing reactions without the addition of the AMV reverse transcriptase. Positive controls were obtained by using genomic DNA as the template.

Protein samples, gel electrophoresis, and immunoblot analysis. Bacterial cells were grown in TrB or TrA for 8 h at 37°C. Cells were harvested from the plates by washing the surfaces of agar plates with cold water and were normalized with respect to the OD_{600} of liquid cultures. Cell suspensions were vortexed and centrifuged at 5,000 × *g* for 15 min at 4°C. Flagellar filaments were collected from the supernatants by high-speed centrifugation at 100,000 × *g* for 1 h and suspended in protein sample buffer containing β-mercaptoethanol (37). Bacterial pellets were lysed in the same buffer by heating at 95°C for 5 min. Protein samples were heated at 95°C for 10 min and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Gels were either silver stained (6) or electrotransferred to nitrocellulose and probed with rabbit antibodies to *B. subtilis* flagellin followed by incubation with a secondary antibody conjugated with horseradish peroxidase. The peroxidase activity was visualized by diaminobenzidine colorimetric reaction in accordance with standard procedures (37).

RESULTS

Isolation of a *B. subtilis* hypermotile mutant. A spontaneous hypermotile mutant was selected by spotting the strain PB1831 (*trpC2 pheA1*) in the middle of a motility plate. Following overnight incubation at 37°C, the rim of the growth halo was picked and transferred onto nutrient agar, and single colonies were tested for increased motility, essentially as described by Pooley and Karamata (35). One isolate, referred to as PB5249, was retained and used for subsequent analysis.

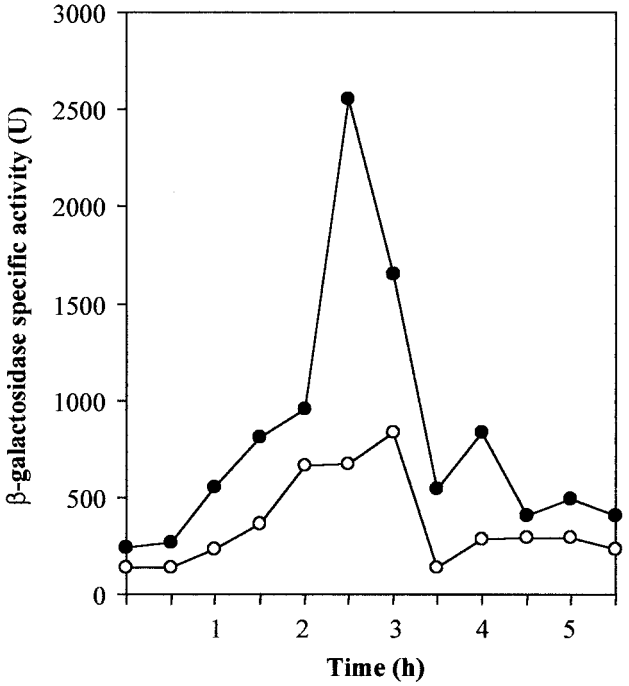

FIG. 1. *hag-lacZ* expression in PB5308 (○) and PB5309 (●). β-Galactosidase activity is expressed in modified Miller units (U) calculated according to the method of Perego and Hoch (34).

The mutation responsible for the hypermotile phenotype was mapped by transformation crosses. *B. subtilis* strains with antibiotic resistance markers present in different positions on the chromosome (38) were used as DNA donors. Competent cells of the mutant were transformed, selected for antibiotic resistance, and screened for motility. The mutation was mapped between *hag* and *uvrA*, at approximately 310° on the *B. subtilis* chromosome. In particular, 80% cotransformation with a resistance marker present in the gene *yvjA* was observed. A more detailed mapping was not attempted. This map position agrees with the previously reported location of an *ifm* mutation (13, 35); thus, the PB5249 mutant was tentatively named *ifmP*.

The increased motility of PB5249 might be explained by an increased number of flagella. This possibility was supported by the observation that the mutant produced more flagellar antigen than the parental strain. In fact, the presence of a flagellum-specific antiserum completely inhibited motility of the parental strain when included in motility agar at a final dilution of 1:10,000. This dilution was ineffective with the mutant strain, which required a 10-fold higher concentration of antiserum (data not shown). A higher amount of flagellin was detected by gel electrophoresis and immunoblotting in supernatants from broth cultures of the mutant than from the parental strain; moreover, flagellin accumulation during growth started 1 h earlier in the mutant than in the parental strain (data not shown). The molecular mass of the flagellin monomer from the mutant was the same as the monomer from the parent; when mixed in a one-to-one ratio and analyzed by MALDI-TOF mass spectrometry, a single peak was found, with an estimated mass of 32,540 Da (calculated mass, 32,472 Da).

The increased level of flagellin in the *ifmP* mutant could have different explanations, such as higher transcription rate, increased stability of the protein, or faster assembly of the flagellin monomers. We measured the level of expression of the flagellin gene by using a *hag-lacZ* transcriptional fusion, integrated ectopically into the chromosomes of PB1831 and PB5249, thus generating the strains PB5308 and PB5309, respectively. The kinetics of β-galactosidase synthesis was similar in the two strains (Fig. 1), but the peak value in PB5309 (2,500 U) was about three times the value obtained with the strain PB5308 (850 U). Therefore, the observed difference between PB1831 and PB5249 in the level of flagellin detected by immunoblotting was consistent with an increased level of *hag* gene transcription.

***ifm* and the surface contact response.** On TrA plates, the strains PB1831 and PB5249 produced morphologically distinct colonies. The parental strain produced extremely rough colonies (Fig. 2A), while the colonies produced by PB5249 were less rough and often exhibited a layered, terraced appearance upon aging (Fig. 3A). Microscopic inspection of the colonies showed that the parental strain formed long cell chains preferentially aligned along the major axis and curled (Fig. 2B). The filaments were long, up to 50 μm, and septa were always present (Fig. 2C). It appeared that the whole community was constituted by short (2 to 3 μm), rod-shaped cells that were indistinguishable from those growing in liquid media (Fig. 2D). More striking was the complete absence of cells bearing flagel-

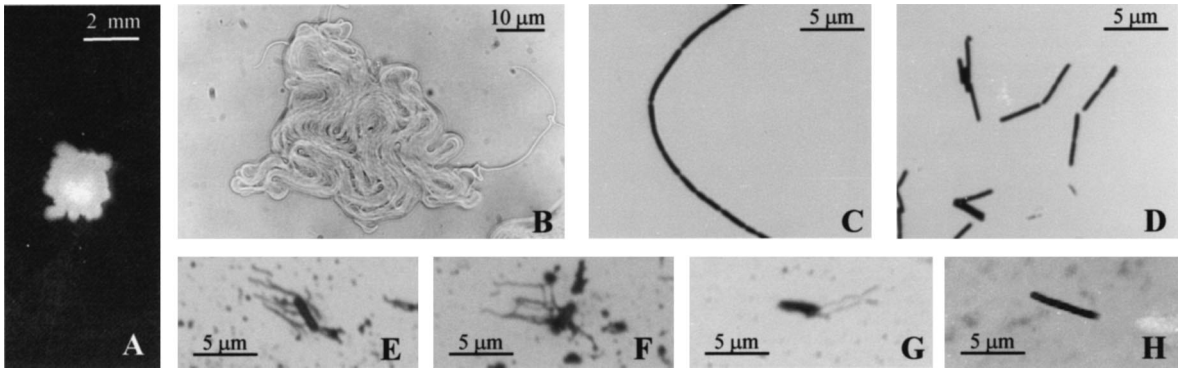

FIG. 2. Morphological traits of *B. subtilis* strain PB1831. Colonies were produced on 1.5% agar plates after incubation for 72 (A) and 8 (B) h at 37°C. Cells taken from colonies (C) and from liquid medium (D) are shown. Also shown are an oligoflagellated cell from liquid culture (E) and cells grown on 0.2 (F), 0.3 (G), and 0.5% (H) agar plates.

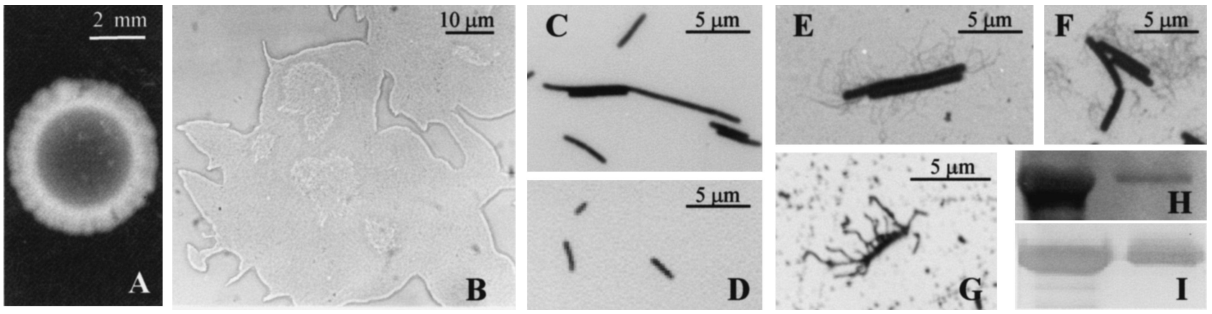

FIG. 3. Morphological and molecular traits of *B. subtilis* strain PB5249. Colonies were produced on 1.5% agar plates after incubation for 72 (A) and 8 (B) h at 37°C. Elongated (C) and hyperflagellated (E through F) swarm cells, short (D) and oligoflagellated (G) cells from liquid medium, and SDS-PAGE (H) and immunoblot with an anti-flagellin antiserum (I) of extracellular flagellin from cells grown in solid (left) and liquid (right) medium are shown

lar filaments at every stage of colony development. Flagella were never viewed at agar concentrations ranging from 2 to 0.5% (Fig. 2H); however, the ability of PB1831 to produce flagella in liquid medium (Fig. 2E) was retained at agar concentrations lower than or equal to 0.3% (Fig. 2F and G). Of great interest was the observation that this behavior was not peculiar to PB1831 but was shared by all the *B. subtilis* strains carrying a wild-type *ifm* locus, such as the reference strain 168 (PB168) and two of its oldest derivatives, PB25 and PB1424 (Table 1). Cells taken from the *ifmP* mutant colonies (Fig. 3B) were longer (8 to 16 μm long) (Fig. 3C) than cells grown in liquid medium (Fig. 3D) and were without septa. Moreover, an increased number of flagella was seen on elongated cells (Fig. 3E and F) in comparison with that for the oligoflagellated cells grown in liquid media (Fig. 3G). Flagella of the hyperflagellated cells appeared to be very fragile, since large amounts of flagellar filaments were very often observed detached from cells in stained preparations (Fig. 3F). The finding that the strain PB5249 produces elongated and hyperflagellated cells when propagated over the surface of solid media demonstrates that *B. subtilis* is provided with the ability to undergo swarming differentiation. Swarming by PB5249 occurred at a wide range of temperatures (20 to 37°C) and on different solid media (TrA, nutrient, and LB agar). Macroscopically, *B. subtilis* swarm colonies did not exhibit regularly layered consolidation phases alternated with swarming migration (Fig. 3A), as has already been reported for other *Bacillus* species (11, 39).

To estimate the extent of surface-induced hyperflagellation in strain PB5249, we measured the differences in the amount of extracellular flagellin in equivalent numbers of cells taken from TrB and TrA by SDS-PAGE and immunoblotting with a *B. subtilis* antiflagellin antiserum. As shown in Fig. 3H, cells grown on the solid medium exhibited an almost 10-fold increase in flagellin compared to those grown in liquid, and the flagellin monomers appeared to have the same molecular weight (Fig. 3I). To confirm the identity of flagellin subunits in swim and swarm cells, a mutant with a deletion of the *hag* gene (PB5250) was constructed by gene replacement with a kanamycin resistance determinant. As expected, the cells failed to swim in liquid media as well as to swarm on solid surfaces, further supporting the finding that the same type of flagellar subunit is employed for the assembly of flagella in swim and swarm cells.

Surfactin is a *B. subtilis* lipopeptide antimicrobial surfactant whose activity has recently been related to the ability of an undomesticated *B. subtilis* strain to swarm (20). Many *B. subtilis* laboratory strains derived from 168, including PB1831, are defective in surfactin biosynthesis due to a frameshift mutation in the *sfp* gene (28). Nevertheless, we tested PB5249 for the ability to produce surfactin. No hemolytic activity was observed on blood agar plates, indicating that the strain is not a surfactin producer. To evaluate whether the swarming behavior of PB5249 was affected by the ability to produce surfactin, we constructed strain PB5332, a surfactin-producing derivative of PB5249. The effect of surfactin production on swarming was limited to a slight increase in the size of colonies (Fig. 4) that did not show alternate cycles of swarming migration and consolidation, as already noted for strain PB5249.

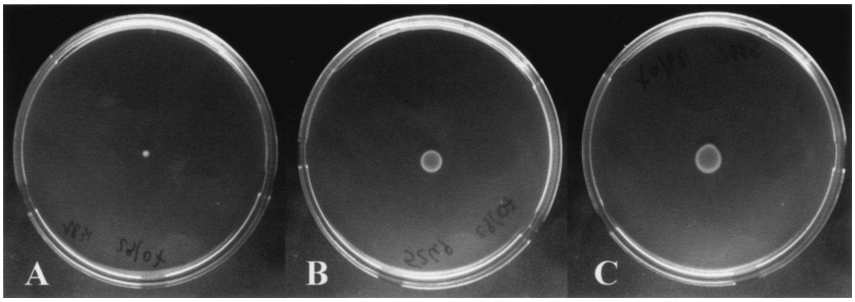

FIG. 4. Effect of the *ifm* mutation and surfactin production on *B. subtilis* colony growth. Growth of the parental strain PB1831 (A), the *ifmP* single mutant PB5249 (B), and the *ifmP sfp* double mutant PB5332 (C) are shown.

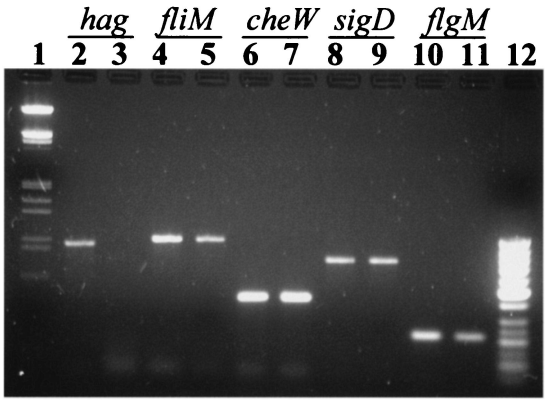

FIG. 5. Transcription of flagellar genes by *B. subtilis* PB1831. Electrophoretic separation of products obtained by RT-PCR on total RNA extracted from PB1831 grown in liquid (lanes 2, 4, 6, 8, 10) or solid (lanes 3, 5, 7, 9, 11) media is shown. Lanes 1 and 12, molecular weight standards.

Transcriptional analysis of flagellar genes in PB1831. The observation that PB1831 failed to undergo surface-induced swarming differentiation and, what is more, failed to produce flagella upon growth on a solid surface induced us to get further insights on the molecular events involved in the response of PB1831 to the solid surface. To this end, the transcription of genes required for flagella formation was analyzed. First, we checked for transcripts of the *hag* class III gene by RT-PCR, and expression of *hag* was never detected when PB1831 was grown on solid media (Fig. 5, lane 3). This result was in accordance with the observation that PB5308 produced white colonies on plates containing X-gal (data not shown).

Since the *hag* gene is transcribed from a σ^D-dependent promoter (25), we analyzed the expression of the *fla*/*che* class II operon that includes the σ^D-encoding gene (*sigD*) by RT-PCRs performed at three different positions along the approximately 26-kb operon. Transcriptional scanning showed that the operon was expressed all over its length and included *sigD* (Fig. 5, lanes 5, 7, 9). Thus, the lack of *hag* expression in solid media could not be interpreted simply as the consequence of a missed transcription of the late-flagellar-stage sigma factor. To understand whether the level of *sigD* expression played a key role in *hag* transcription in strain PB1831 grown on solid surfaces, PB1831 was transformed with the plasmid pSigD, which carries a copy of the *sigD* gene under control of the IPTG (isopropyl-β-D-thiogalactopyranoside)-inducible P_{spac} promoter (3). PB1831 harboring pSigD restored the ability to produce flagellin when grown on solid agar plates in the presence of the inducer (Fig. 6; lane 2). This result demonstrates that the level of intracellular σ^D in PB1831 propagated on solid surfaces is not sufficiently high to promote *hag* expression.

The activity of σ^D is intracellularly balanced by the anti-sigma factor FlgM (10). Therefore, experiments were performed to evaluate if on solid media (i) the *flgM* was transcribed in PB1831 and (ii) flagellin was synthesized in a PB1831 derivative carrying a deletion in *flgM* (PB5148). A positive signal was obtained by RT-PCR for the *flgM* gene in cells of strain PB1831 (Fig. 5, lane 11), and flagellin synthesis was restored in strain PB5148, as demonstrated by immunoblot analysis (Fig. 6, lane 4). These results show that intracellular FlgM can potentially be responsible for σ^D inactivity and, consequently, for missed *hag* expression in strain PB1831 grown on solid media. However, cells overexpressing the transcription factor σ^D and cells lacking FlgM recovered only part of their biosynthetic potential, since they did not acquire the ability to produce flagella when grown on solid media. The lack of flagella in PB1831 suggests that the *ifmP* locus regulates the complex process of flagella formation in response to external stimuli coming from liquid or solid environments at a not-yet-defined level.

DISCUSSION

Bacterial flagella are organelles of the cell envelope instrumental to interaction with the environment. Flagellum-driven motility is one of the most impressive features in the cellular physiology of eubacteria and allows active bacterial movement in liquid as well as over solid surfaces. In the environment, the ability to swim enables individual cells to rapidly respond to changes in nutrient availability, moving toward attractants or away from repellents through a signal transduction chemotactic network. In contrast to swimming, the movement of flagellated bacteria over solid surfaces is a striking multicellular behavior, which enables bacterial cells to move collectively in a coordinated fashion referred to as swarming motility (17). Swarming, which is not triggered by starvation but by contact with solid surfaces, closely depends on the ability of swimmer cells to undergo a surface-induced differentiation process leading to the production of elongated and hyperflagellated swarm cells (8, 15, 17, 19, 39). Swarming over solid surfaces is a way for bacteria to disperse and to colonize the environment that favors the establishment of commensal, symbiotic, or pathogenic associations with plants and animals to reach optimal colonization niches (27, 32).

B. subtilis is a flagellated soil bacterium ubiquitously distributed in the environment. It possesses a remarkable metabolic and physiological versatility that facilitates its propagation in a wide range of growth conditions, including liquid and solid substrates. As with other flagellated bacteria, the ability to alternate swimming with swarming motility in response to a surface stimulus may be of evident adaptive value for this organism. In this report, we show that swarming is not a widespread behavior of *B. subtilis* laboratory strains but can be observed only in a strain exhibiting a hypermotile phenotype due to a mutation in the *ifm* locus.

The hypermotile *ifmP* mutant responds to contact with a solid surface similarly to other *Bacillus* species (11, 20, 39). The swarm colonies produced by the *ifmP* mutant only occasionally had a terraced appearance and never exhibited the consolida-

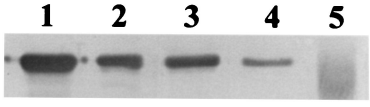

FIG. 6. Immunoblot analysis of flagellin production by PB1831 pSIGD (lanes 1 and 2) and PB5148 (*flgMΔ80*) (lanes 3 and 4) grown in liquid (lanes 1 and 3) or solid (lanes 2 and 4) media. Lane 5, molecular weight standard.

tion phases that are peculiar to *Proteus mirabilis* (5, 17). *B. subtilis* swarm cells are 3 to 5 times longer and almost 10 times more flagellated than the swimmer cells. The *ifmP* mutant was unable to produce surfactin, which has been shown to play a role in *B. subtilis* swarming (20). In this study, we provide evidence that surfactin facilitates bacterial migration over solid surfaces but is not essential for swarming differentiation in an *ifmP* background.

The same motile organelle appears to be required for swimming and swarming movements in *B. subtilis*. Indeed, a flagellin monomer of the same molecular weight is assembled by *B. subtilis* grown in liquid or on solid media, and disruption of the *hag* gene abolishes surface translocation. This phenomenon is similar to that found in some members of the *Enterobacteriaceae* (15, 41) but differs from that observed in *Vibrio parahaemolyticus*, which produces distinct flagellar organelles for swimming and swarming motility (24).

Integrity in the expression of flagellar and chemotaxis genes is an essential requirement for swimming and swarming motility in several gram-negative and gram-positive bacteria (5, 7, 11, 17, 20, 31, 39, 41). That a flagellar locus can influence the ability to generate flagella only upon contact with a solid surface has never been described before, however. Indeed, the parental strain and all the strains carrying a wild-type *ifm* locus not only failed to activate a swarm response upon contact with the surface but, remarkably, completely stopped synthesizing flagellin and assembling flagellar filaments. The lack of *hag* gene expression can be explained at the regulatory level by a functional deficiency of the transcription factor σ^D. This interpretation is supported by the results obtained by overproduction of σ^D in the presence of the plasmid pSigD or by deletion of the *flgM* gene encoding the anti-sigma factor FlgM. In both experimental systems, cells grown on solid surfaces succeeded in expressing flagellin; nevertheless, the ability to assemble flagellar filaments was not restored.

Thus, in strains having a wild-type *ifm* background, some of the steps required for the integration of signals derived from contact with a solid surface are missing or not functioning properly. The *ifmP* mutation, therefore, can be regarded as a gain of a mutated function, restoring a response lost in the process of domestication of *B. subtilis*.

The presence of a mutant *ifm* gene appears to be necessary to correctly perceive the solid-surface signal in order to process and integrate it into the different regulatory pathways that affect flagellar assembly and functions.

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