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Characterization of a Novel Gene, *wosA*, Regulating FlhDC Expression in *Proteus mirabilis*[▽]

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In this study, we describe *wosA*, a *Proteus mirabilis* gene identified by its ability to increase swarming motility when overexpressed. At various times during the swarming cycle, the increased expression of *wosA* resulted in a 4- to 16-fold upregulation of the transcription of *flhDC*, encoding the master regulator of the flagellar cascade. In turn, the expression of *flaA*, encoding flagellin, was substantially increased in *wosA*-overexpressing strains. The overexpression of *wosA* also resulted in constitutive swarmer cell differentiation in liquid medium, a normally nonpermissive condition. However, in *wosA*-overexpressing strains, the onset of swarming was not altered. A null *wosA* allele resulted in a slight decrease in swarming motility. The expression of *wosA* was growth phase dependent during growth in liquid and on agar plates during swarmer cell differentiation. Increasing the viscosity of liquid medium by the addition of polyvinylpyrrolidone induced swarmer cell differentiation and resulted in a fourfold increase in *wosA* transcription. A *fliL* mutation that results in constitutive swarmer cell elongation also increased *wosA* transcription. In this study, we discuss the possible role of the *wosA* gene product in signal transduction from solid surfaces to induce swarmer cell differentiation, possibly via alterations in the motor switch complex. This study also suggests that despite constitutive swarmer cell differentiation in *wosA*-overexpressing strains, there are additional regulatory and/or environmental conditions that may control the onset of swarming migration.

Proteus mirabilis is a motile gram-negative bacterium that undergoes cellular differentiation and a coordinated social behavior termed swarming motility that results in movement over solid surfaces (reviewed in references 3, 15, and 36). During differentiation, short rod-shaped vegetative cells with few peritrichous flagella elongate 20- to 40-fold and increase the number of flagella by >50-fold to form swarmer cells. Swarming then occurs as cellular differentiation, migration away from a central inoculation point, and consolidation or a period of dedifferentiation to produce terraces in a characteristic pattern of growth on 1.5 to 2% agar (15, 37). The process of differentiation depends on certain environmental conditions, including a solid surface and the inhibition of flagellar rotation (1, 15, 37). In addition, although not well understood, extracellular signaling has been implicated in the process of swarmer cell differentiation (4, 43).

In *Escherichia coli* and *Salmonella enterica* serovar Typhimurium, the production of flagella requires the expression of more than 50 genes that are coordinately regulated in a regulon comprised of three temporally regulated hierarchical transcriptional classes: class I, class II, and class III. The heterotetrameric $FlhD_2C_2$ master regulator of the flagellar gene hierarchy is encoded by the class I operon *flhDC*. The transcription of all the class II and III genes of the flagellar cascade is dependent on the expression of *flhDC* (reviewed in reference 10). In *E. coli* and *S. enterica* serovar Typhimurium, the $FlhD_2C_2$ regulator acts to integrate a variety of regulatory inputs to control the cascade of flagellar gene expression. These inputs include catabolite repression (41, 46), osmolarity (40), heat shock (39), acetyl phosphate (40), and cell cycle regulation (33). The regulation of swarmer cell differentiation in *P. mirabilis* and other swarming bacteria has also been shown previously to act through $FlhD_2C_2$ (12, 14, 16, 17, 42, 44, 47). One hallmark of the switch to a swarmer cell from a vegetatively growing swimmer cell is a sharp transient increase in the expression of *flhDC* (11). The increased FlhDC levels then ultimately lead to a drastic increase in the expression of the class III flagellar gene *flaA* that encodes the monomer subunit of the flagellar filament, resulting in hyperflagellation of the swarmer cell (15). The artificial overexpression of *flhDC* in both *P. mirabilis* and another swarming bacterium, *Serratia liquefaciens*, causes increased elongation and hyperflagellation, with a corresponding increase in both the rapidity of onset and the velocity of swarming (12, 16). Moreover, *E. coli* FlhD has been shown previously to suppress cell division (35). Multiple *P. mirabilis* mutations that affect *flhDC* transcription and, therefore, swarming have been identified. Mutations of the global transcriptional regulator leucine-responsive protein (encoded by *lrp*) (21) and the four novel regulators encoded by *umoA* to *umoD* (11) that normally upregulate *flhDC* transcription prevent or cause defective swarmer cell differentiation. The RcsCDB phosphorelay protein, originally identified as a positive regulator of capsular polysaccharide synthesis in *E. coli* (reviewed in reference 28), has also been shown previously to repress the transcription of the *flhDC* operon in *E. coli* (14). The *P. mirabilis* homolog of RcsD is the RsbA protein (7, 27). Mutations in *rsbA* lead to a hyperswarming, or "precocious," phenotype likely caused by the derepression of *flhDC* expression (7, 27).

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description and/or relevant genotype	Source or reference
E. coli strains		
DH5α	F$^-$ ϕ80d*lacZ*Δ*M15* Δ(*lacZYA-argF′*)*U169 endA1 recA1 hsdR17*(r_K^- m_K^-) *deoR thi-1 supE44* λ$^-$ *gyrA96 relA1*	Laboratory stock
SM10 λpir	*thi thr leu tonA lacY supE recA* RP4-2Tc::Mu Km^r λ*pir*	32
P. mirabilis strains		
PM7002	Wild type; Tc^r	ATCC
PM437	*speA*::mini-Tn*5lacZ1* Tc^r	43
BB2000	Wild type; Rf^r Tc^r	6
BB2204	BB2000 *fliL2204*::mini-Tn*5*-Cm Cm^r Rf^r Tc^r; non-wild-type elongation	9
PM7002 *wosA*::pKNG101 *wosA*	Sm^r Tc^r	This study
PM7002 *wosA*::pKNG101 *wosA-lacZ*	Sm^r Tc^r	This study
Plasmids		
pBC SK$^-$	High copy no.; Cm^r	Stratagene
pKNG101	R6K-derived suicide vector; Sm^r	25
pQF50	Amp^r	13
pSwarm7	High copy no.; Cm^r	This study
pORF1	High copy no.; Cm^r	This study
p$YbhK_{Pm}$	High copy no.; Cm^r	This study
pKNG101 *wosA*	Sm^r	This study
pKNG101 *wosA-lacZ*	Sm^r	This study

Many bacteria, including *Escherichia*, *Salmonella*, *Bacillus*, *Proteus*, *Serratia*, and *Vibrio* species, undergo swarming or surface-induced motility (2, 20, 22, 45). Swarmer cell differentiation is initiated upon surface contact and typically involves the expression of peritrichous flagella and the elongation of a vegetatively growing cell. In *Vibrio parahaemolyticus*, the restriction of the rotation of the polar flagellum by the addition of anti-polar flagellum antiserum to the culture medium (8) or by an increase in the medium's viscosity (30), as well as plating onto a solid agar surface, results in the expression of the *laf* genes which encode components of the lateral flagella used for swarming motility. The polar flagellum therefore acts as a mechanosensor that senses a decrease in flagellar rotation and subsequently directs *laf* gene expression (26). In *P. mirabilis*, the molecular signals for swarming are still not well understood. However, the same conditions that inhibit flagellar rotation in *V. parahaemolyticus* to cause swarmer cell differentiation in normally noninducing liquid medium (with the addition of anti-flagellum antiserum and with high viscosity) also cause swarmer cell differentiation in *P. mirabilis* (9). Moreover, mutations in the *P. mirabilis* genes *fliL*, *fliF*, and *fliG*, which encode components of the flagellar basal body, confer a defective swarming phenotype yet result in swarmer cell differentiation in normally noninducing liquid medium and hyperelongated swarmer cells on a solid agar surface. This finding again implicates the rotating flagellar filament as being critical for signal sensing during the process of swarmer cell differentiation (9).

In this work, we describe the gene *wosA*, which causes a novel swarming phenotype when overexpressed in wild-type *P. mirabilis*. *wosA* overexpression caused constitutive swarmer cell differentiation under noninducing conditions and hyperswarming despite maintaining the same pattern of onset of swarming migration as that of the wild-type strain. The hyperswarming phenotype is likely due to (i) increased swarming velocity and (ii) the overexpression of *flhDC* throughout the swarm cycle. A null allele of *wosA* resulted in a slight decrease in swarming motility. Interestingly, the inhibition of flagellar rotation by an increase in the medium viscosity or by a mutation in the *fliL* gene led to the upregulation of *wosA* expression. Our results indicate that *wosA* is involved in the regulation of *flhDC* expression but also suggest that *wosA* may function in a signaling pathway from the flagellar motor switch complex. Moreover, our results suggest that additional regulation exists to control swarmer cell differentiation and the onset of swarming migration.

MATERIALS AND METHODS

Strains, plasmids, and media. The bacterial strains and plasmids used in this work are listed in Table 1. Routine microbiological procedures and enzymatic manipulations of DNA were carried out by standard methods (38). Both *E. coli* and *P. mirabilis* strains were maintained in Luria-Bertani (LB) broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter of distilled water) or on LB with agar at a concentration of 1.5 to 2.0%. The concentration of antibiotics used for the selection of *E. coli* on LB agar and in culture was 25 μg/ml for both chloramphenicol and streptomycin. Antibiotic concentrations used for the selection of *P. mirabilis* on LB agar and in culture were 100 μg/ml for chloramphenicol, 35 μg/ml for streptomycin, and 15 μg/ml for tetracycline. X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) in *N*,*N*-dimethylformamide was used at a final concentration of 100 μg/ml in indicator agar.

Strain construction. The pSwarm7 plasmid was digested with HpaII, and a 5.1-kb fragment consisting of the pBC SK$^-$ vector and *orf1* was recircularized to form the *orf1* plasmid. This plasmid contains the full 274-bp sequence upstream of *orf1* (*wosA*) that includes the putative *wosA* promoter and 500 bp downstream of the gene found in the original pSwarm7 plasmid. The *P. mirabilis ybhK* ($ybhK_{Pm}$) gene was isolated on a 1.1-kb DraI fragment and ligated into the EcoRV site of pBC SK$^-$. Both plasmids were introduced into wild-type *P. mirabilis* PM7002 by electroporation using standard procedures. The transcription of $ybhK_{Pm}$ results from the *lac* promoter found on the pBC SK$^-$ cloning vector.

The disruption of *wosA* was accomplished by homologous recombination between the chromosomal copy of *wosA* and a 400-bp internal fragment of *wosA*, PCR amplified using the 5′-ATGCTCTAGAACCAATGACTTTTACCAGTTA C-3′ and 5′-GGTACTCGAGCGACATCGGAAAATGTTTGATG-3′ primers

incorporating XbaI and XhoI restriction sites and cloned into the XbaI- and SalI-digested suicide vector pKNG101 (25). Southern blot analysis confirmed the disruption of the *wosA* locus (data not shown).

T7 and T3 primers were used to amplify *wosA* from the pWosA plasmid. The 1,493-bp PCR product was then gel purified and digested with XbaI and HindIII to produce an 844-bp fragment containing 274 bp upstream of *wosA* and 570 bp of the *wosA* gene. This fragment was then ligated into the XbaI- and HindIII-digested promoter probe vector pQF50 (13) to give the pQF50*wosA-lacZ* promoter fusion plasmid. The *wosA-lacZ* fusion was then isolated on an XbaI-ScaI fragment from the pQF50*wosA-lacZ* plasmid and ligated into the XbaI- and SmaI-digested suicide vector pKNG101 (25). Single-copy *wosA-lacZ* fusions were constructed in wild-type and *wosA*::Km^r strains by homologous recombination between the chromosomal copy of *wosA* and the *wosA-lacZ* fusion ligated into pKNG101 (25). Southern blotting and PCR analysis confirmed the correct insertion at the *wosA* locus (data not shown).

Swarmer cell elongation and swarming migration assays. Strains were grown overnight at 37°C in LB medium containing appropriate antibiotics with or without shaking. Cultures were diluted to the same optical density at 600 nm (OD_{600}), 4 μl of each culture was spotted onto 1.5 or 2.0% LB agar, and the culture plates were incubated at 37°C. Swarmer cell elongation was determined by examining cells from the peripheries of the swarming colonies by phase-contrast microscopy. Because cell samples from the surface of an agar plate are often made up of mixed populations of both swimmer cells and swarmer cells, for this study, any cell with a length ≥5 times the mean length of a swimmer cell (1.96 ± 0.30 μm) was defined as a swarmer cell. At least 100 total cells were measured to determine the percentage of swarmer cells in the sample, and at least 50 swarmer cells were measured to determine the mean swarmer cell length, reported as the mean ± the standard deviation (SD). Swarming diameter was measured every 30 min to determine migration distance.

Northern blot analyses. To assay *flhDC*, *flaA*, or *wosA* mRNA levels, relevant strains were grown overnight in LB medium containing appropriate antibiotics and used to inoculate fresh media, and cultures were grown with or without shaking at 37°C to a specified OD_{600}. Two hundred-microliter aliquots of the cultures were then plated onto several LB agar plates in parallel to produce synchronously differentiating plate cultures. RNA was prepared from cells harvested from these plates in Tris-EDTA at 1-h intervals throughout the swarm cycle (1 h postinoculation of the plates [T_1] to T_6). One milliliter of a culture at a specified OD_{600} in liquid medium (LB) was also harvested for RNA extraction. Total RNA was prepared from each sample using the Masterpure RNA purification kit (Epicenter). Northern blot analysis was carried out with equivalent amounts of formamide-formaldehyde-denatured RNA electrophoretically separated through a 1.2% formaldehyde agarose gel and transferred onto a nylon membrane (Magnagraph; GE Osmonics) by downward capillary transfer. Blots were probed with a digoxigenin-labeled PCR-amplified segment of either the *flhDC* gene (5′-TCGGACGGGATGTAAAGAGA-3′ and 5′-CAGGATTGGC GGAAAGTTTA-3′), the *flaA* gene (5′-TATCTGGGGTGCCGATAAAC-3′ and 5′-ACGGTTTTGAATCGCACCTA-3′), or the *wosA* gene (5′-ATGCTCT AGAACCAATGACTTTTACCAGTTAC-3′ and 5′-GGTACTCGAGCGACA TCGGAAAATGTTTGATG-3′) from *P. mirabilis* PM7002. Transcripts were visualized using the CDP-Star substrate according to the instructions of the manufacturer (Roche Applied Science).

Cell growth and measurement of *wosA* gene expression using a *wosA-lacZ* transcriptional fusion. For samples from liquid culture, an overnight culture of the PM7002 *wosA*::*wosA-lacZ* strain in LB containing streptomycin was grown at room temperature without shaking to produce a low-density inoculum. The next morning, 30 ml of LB containing streptomycin was inoculated at a 1:1,000 dilution of the overnight culture. The cells were then incubated at 37°C with shaking, and 0.9-ml samples at various OD_{600}s were collected in duplicate. The β-galactosidase activity of sodium dodecyl sulfate-chloroform-treated cells was assayed using the substrate *ortho*-nitrophenol-β-D-galactopyranoside by the method of Miller (31), and the results were reported as the means ± SDs.

To assay β-galactosidase activity from swarmer cells during a swarm cycle, an overnight culture of the PM7002 *wosA*::*wosA-lacZ* strain in LB containing streptomycin was grown at 37°C without shaking. The next morning, 30 ml of LB containing streptomycin was inoculated at a 1:200 dilution of the overnight culture. The cells were then incubated at 37°C with shaking to an OD_{600} of 0.4, and 200-μl samples were plated in parallel onto multiple plates to produce synchronously differentiated cells. Plates were harvested in phosphate-buffered saline, pH 7.4, at 1-h intervals, the OD_{600} at each time point was determined, and 0.9-ml samples of cells were collected in duplicate for analysis. β-galactosidase activity was assayed as described above for liquid culture samples.

For conditioned-medium preparations, 30-ml aliquots of LB broth cultures were used. Cells were grown to an OD_{600} of 1.2, and spent medium was collected by pelleting the cells at 4,300 × *g* for 10 min. Cell-free medium was prepared by adjusting the pH to 7.5 and filter sterilizing with a 0.22-μm-pore-size filter unit (Nalgene). The first 5 ml was discarded to wash off filter components. Individual cultures for the assay of β-galactosidase activity were grown in conditioned medium at 37°C with shaking, and 0.9-ml samples at various OD_{600}s were collected in duplicate. β-galactosidase activity was assayed as described in the previous paragraphs.

Preparation of PVP and cell growth in PVP. To increase the viscosity of LB medium, polyvinylpyrrolidone 360 (PVP; Sigma) was dissolved to a concentration of 30% in distilled water. This solution was dialyzed overnight against five changes of 100 volumes of distilled water. The volume of the PVP solution doubled upon dialysis, resulting in a concentration of 15% PVP. LB solids were added directly to the dialyzed PVP solution, and the solution was sterilized, resulting in LB medium containing 15% PVP. For growth in PVP, vegetative *P. mirabilis* cells were harvested by centrifugation of an overnight LB broth culture grown at 37°C without shaking and were resuspended in fresh broth to an OD_{600} of 0.6. The washed cells were inoculated at a dilution of 1:100 into both LB and LB medium containing 15% PVP and the appropriate antibiotics and grown at 37°C with shaking. After 2 h of growth, cells were harvested at 30-min intervals for the assay of β-galactosidase activity. β-galactosidase activity was assayed as described above.

Nucleotide sequence accession number. The *orf1* (*wosA*) nucleotide sequence determined in this study has been deposited in GenBank under accession no. EU164546.

RESULTS

Identification of pSwarm7, a multicopy suppressor of a swarming-defective *speA* mutant. Previous work identified a putrescine biosynthetic pathway mutant, PM437 (*speA*::mini-Tn*5lacZ1*), that was defective in swarmer cell differentiation and, thus, swarming motility (43). The addition of exogenous putrescine restored the swarming motility of this mutant to wild-type levels. In an attempt to identify multicopy suppressors of the defect in the putrescine signaling pathway, PM437 was electroporated with a pBC SK$^-$ library of partial Sau3AI-digested chromosomal DNA from the PM437 strain. pSwarm7 was identified as a plasmid that restored the swarming phenotype to PM437 (Fig. 1A). However, the restoration of the swarming phenotype appeared to be independent of the *speA* mutation, as wild-type *P. mirabilis* PM7002 transformed with the pSwarm7 plasmid also exhibited increased swarming (Fig. 1A).

The sequencing of the pSwarm7 clone revealed the presence of two divergently transcribed open reading frames (ORFs) in the cloned insert. Neither of the two ORFs encoded products of known function, as determined by comparing the ORF sequences to sequences in GenBank. *orf1* encodes a putative 321-residue protein with an unknown function and no significant similarity to other proteins. Interestingly, although the organization of the genes in the genome surrounding *orf1* is conserved between *P. mirabilis* and *E. coli*, a homolog of *orf1* is not present in the *E. coli* genome. The second ORF, a homolog of the *E. coli ybhK* gene, encodes a putative 311-residue protein that belongs to an uncharacterized protein family, UPF0052 (29); we designated this gene $ybhK_{Pm}$.

To determine which gene was responsible for the hyperswarming phenotype, a library of random Tn*7* Kan insertions in pSwarm7 was generated in vitro using the pGPS5 vector from the GPS-LS linker-scanning system (New England Biolabs). Plasmids unable to restore the swarming phenotype to PM437 were sequenced, and it was shown that all insertions that prevented the enhanced swarming phenotype were within *orf1*. Moreover, it was unlikely that polar effects on the $ybhK_{Pm}$

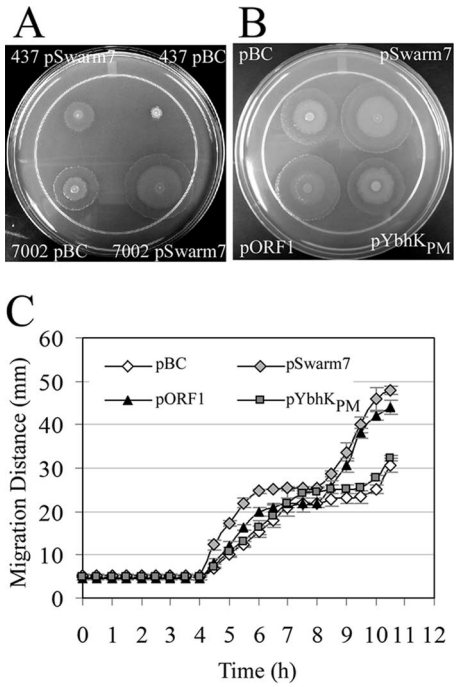

FIG. 1. WosA overexpression increases the swarming motility of *P. mirabilis*. (A) Swarming behavior of wild-type *P. mirabilis* PM7002 and PM437 (*speA*::mini-Tn*5lacZ1*) carrying the cloning vector pBC SK$^-$ or the pSwarm7 plasmid and grown on 2% LB agar at 37°C for 16 h. (B) Comparison of the swarming behavior of wild-type *P. mirabilis* PM7002 carrying cloning vector pBC SK$^-$ or the pSwarm7 plasmid with that of PM7002 containing the pORF1 or pYbhK$_{Pm}$ plasmid. Strains were grown on 1.5% LB agar at 37°C for 10 h. (C) Swarming migration of wild-type *P. mirabilis* 7002 carrying either the pBC vector alone or the pSwarm7, pORF1, or pYbhK$_{Pm}$ plasmid. Strains were grown overnight at 37°C without shaking in 2.5 ml of LB supplemented with 100 μg of chloramphenicol/ml. Cultures were diluted to the same OD_{600} (0.4), 4 μl of each culture was spotted onto 1.5% LB agar with appropriate antibiotics, and swarming diameter was measured every 30 min over a 10.5-h period. Values are the means of results from three independent experiments. Error bars, SDs.

gene were responsible for the lack of enhanced swarming in these strains, as the *ybhK$_{Pm}$* gene and *orf1* are transcribed divergently.

To independently verify that *orf1* was the gene responsible for the phenotype observed in the pSwarm7 overexpression strain, both *orf1* and *ybhK$_{Pm}$* were individually subcloned into the pBC SK$^-$ multicopy cloning vector. The swarming phenotype and the migration of the strains containing each of the genes were evaluated in comparison to those of wild-type PM7002 containing vector alone and PM7002 containing the original pSwarm7 plasmid (Fig. 1B and C). All four strains examined initiated swarming at 4 h after plating. Both PM7002(pSwarm7) and PM7002(pORF1) displayed a hyperswarming phenotype relative to wild-type PM7002(pBC SK$^-$), with swarming velocities of approximately 10, 8, and 6 mm/h, respectively. PM7002(pYbhK$_{Pm}$), however, did not show enhanced swarming relative to the wild-type strain. Interestingly, although the patterns of the onset of swarming were the same for all four strains, the pSwarm7 and pORF1 strains appeared to have a shorter swarming period of 2 h (hours 4 to 6 postplating). Wild-type and pYbhK$_{Pm}$ strains continued swarming for an additional hour. Moreover, the pSwarm7 and pORF1 strains remained in consolidation for only 2 h, while the wild-type and pYbhK$_{Pm}$ strains were in consolidation for 2.5 h. Indeed, if we define consolidation by a change of less than 10% in migration distance during a 1/2-h period, then the four strains showed significantly different consolidation times (analysis of variance [ANOVA]: $F_{3,8} = 22.22$; $P = 0.0003$). We used Tukey's honestly significantly different test (34) to determine which consolidation times differed significantly ($P < 0.05$) from each other and found that the consolidation times of approximately 2 h for the pSwarm7 and pORF1 strains did not differ significantly from each other but did differ significantly from those for the wild-type and pYbhK$_{Pm}$ strains, which were in consolidation for ~2.5 h. This pattern of swarming migration of the pSwarm7 and pORF1 strains resulted in larger swarming diameters than those for the wild-type or pYbhK$_{Pm}$ strain and was reproducible when examined upon multiple occasions. Similar effects on swimming-area diameters for the strains on 0.3% LB agar were observed when swimming motility was examined (data not shown). These results suggested that the overexpression of *orf1* confers the swarming phenotype observed for the pSwarm7 overexpression strain. Thus, this ORF was designated *wosA* (*w*ild-type *o*nset with *s*uperswarming) and the pORF1 plasmid was designated pWosA.

***wosA* is a gene predicted to encode a membrane-associated protein.** The *wosA* gene encodes a putative protein consisting of 321 residues with a calculated molecular mass of 36.91 kDa and an isoelectric point (pI) of 4.44. The *wosA* gene is immediately adjacent to the *uvrB* and *ybhK$_{Pm}$* genes on the *P. mirabilis* chromosome. Upstream of the *uvrB* gene is the biotin biosynthesis operon, and downstream of the *ybhK$_{Pm}$* gene is the molybdopterin biosynthesis operon. This genome organization corresponds to that found in *E. coli* except for the absence of the *wosA* gene from the *E. coli* genome. The *wosA* gene is not part of an operon, and the WosA protein has no significant identity to any proteins of known function but shows 27% identity over 185 residues to a hypothetical protein, HCH 06118, from the marine bacterium *Hahella chejuensis* KCTC 2396 (24). The WosA protein is predicted to have a single transmembrane domain and is oriented such that the N-terminal 6 to 29 residues span the cell membrane while the remainder of the protein is found in the cytoplasm (23). No other protein motifs were identified.

The overexpression of *wosA* causes swarmer cell differentiation under noninducing conditions. In cells containing pWosA, the level of *wosA* overexpression was >50-fold above that in cells containing vector only as determined by Northern analyses (data not shown). Swarming migration assays with the PM7002(pWosA) strain [PM7002(pORF1)] indicated that the onset of swarming was the same as that for wild-type PM7002 (Fig. 1C). Interestingly, PM7002(pWosA) exhibited what appeared to be constitutive swarmer cell differentiation during growth in liquid culture and at 2 h after plating onto LB agar (T_2), during the early stages of synchronous growth (Fig. 2). The overexpression of the *ybhK$_{Pm}$* gene did not induce swarmer cell differentiation under the same conditions (data not shown). At 5 h after plating (Fig. 2, row T_5), both wild-type and pWosA strains showed the presence of swarmer cells; however, the swarmer cells of the pWosA strain were significantly longer and more abundant than those of the wild-type

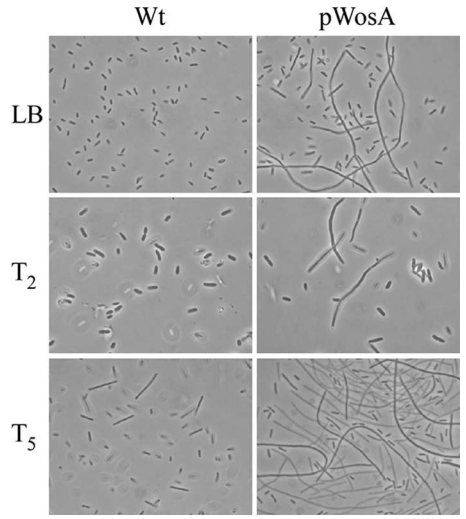

FIG. 2. Effect of *wosA* overexpression on the differentiation of wild-type *P. mirabilis* 7002 cells. (Top row) Strains were grown in LB medium at 37°C with shaking, harvested at an OD_{600} of 1.2, and examined by phase-contrast microscopy. (Middle and bottom rows) Overnight cultures of wild-type (Wt) and pWosA strains were adjusted to the same OD and seeded onto LB agar plates, and the plates were incubated at 37°C to produce synchronous cultures. At time points of 2 h (T_2) and 5 h (T_5) after seeding, the cells were washed from agar plates and examined by phase-contrast microscopy.

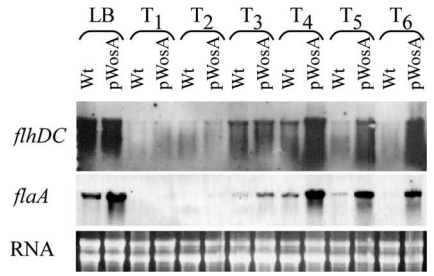

FIG. 3. Effect of *wosA* overexpression on *flhDC* and *flaA* transcription. Northern hybridization of *flhDC* (top panel) and *flaA* (middle panel) probes to total RNA from wild-type (Wt) PM7002 carrying the pBC SK^- vector alone and PM7002 carrying the pWosA plasmid. Strain samples were taken from the liquid inoculum at an OD_{600} of 0.6 (LB) and from synchronous plate cultures at 1-h intervals over a swarmer cell differentiation cycle (T_1 to T_6). For each Northern blot, the same preparation of RNA was used to allow for the direct comparison of different transcripts during the swarm cycle. A representative ethidium bromide-stained gel containing the same amount of RNA used in each Northern blot is shown in the bottom panel.

strain. Forty percent of the cells of the wild-type strain carrying pBC differentiated into swarmer cells, with a mean cell length of 13.83 ± 3.53 μm, and ~70% of the cells of the strain carrying pWosA were swarmer cells, with a mean cell length of 23.6 ± 15.58 μm (ANOVA: $F_{1,194} = 19.14$; $P = 0.00002$). In addition, swarmer cells of the strains carrying pWosA varied greatly in size and could reach >100 μm in length. The effect of overexpressing *wosA* was specific to *P. mirabilis*, as the introduction of the pWosA plasmid into *E. coli* did not induce swarmer cell differentiation in *E. coli* (data not shown).

The overexpression of *wosA* causes the upregulation of *flhDC* and *flaA* transcript levels. In *P. mirabilis*, the master operon *flhDC* of the flagellar gene hierarchy is strongly induced during swarmer cell differentiation and the artificial overexpression of *flhDC* increases elongation and hyperflagellation, resulting in the early onset of swarming and greater swarming velocity (16). To determine if the overexpression of *wosA* resulted in the upregulation of genes in the flagellar hierarchy, levels of *flhDC* and *flaA* transcripts were assayed by Northern blotting (Fig. 3). In both PM7002 containing the vector control and PM7002 containing pWosA, the levels of *flhDC* transcripts began to increase at 3 h after plating (T_3), with a greater increase at T_4 (Fig. 3, top panel). However, in PM7002 containing pWosA, the levels of *flhDC* at T_4 were approximately fourfold higher than those in cells containing the vector only, as determined by comparisons of hybridization intensities of diluted RNA samples (data not shown). At T_5 and T_6, when cells were in consolidation, *flhDC* mRNA levels in cells with pWosA were 8- and 16-fold, respectively, above the levels in cells with the vector only (Fig. 3). The levels of *flhDC* transcripts in samples from cultures in liquid medium (LB) taken at the mid-log phase of growth did not appear to differ significantly between wild-type PM7002 carrying the pBC SK^- vector and PM7002 carrying the pWosA plasmid. The *flaA* mRNA levels, however, suggest that there may have been a subtle increase in *flhDC* transcript levels, as we saw a clear and significant 2.2-fold increase in *flaA* mRNA levels in the pWosA culture sample relative to the levels in the wild-type culture (in LB) (Fig. 3, middle panel). Western blot analysis of flagellin in liquid-grown cells with pWosA indicated that the levels of flagellin were at least threefold higher than those in vector-containing cells (data not shown). These results indicate that the elongated cells observed in liquid culture were likely to be differentiated swarmer cells. Moreover, the effect of increased *flhDC* expression due to the overexpression of *wosA* was specific to *P. mirabilis*. The introduction of the pWosA plasmid into the *E. coli* strain FDCF7789 carrying a *flhDC-lacZ* translational fusion produced no increase in *flhDC* expression, as demonstrated when cells were harvested from fronts of swarming *E. coli* cells on Eiken agar and compared to cells of the same strain carrying the pBC vector (data not shown).

The expression of *flaA* during the swarming cycle in cells containing vector only and in cells containing pWosA was also examined (Fig. 3). Although not apparent from the exposure shown, *flaA* transcripts were present in the pWosA strain throughout the early stages of the swarm cycle (T_1 to T_2), albeit at much lower levels than in later stages (T_3 to T_6). No *flaA* transcript was observed in the wild-type strain at T_1 and T_2, even after extended film exposure (data not shown). From T_3 to T_6 in the swarm cycle, cells with pWosA exhibited large differences in *flaA* expression compared to wild-type cells. The increase in *flaA* transcripts in the wild-type strain at T_3 and the subsequent decrease at T_5 correspond to the pattern expected of a swarm cycle. However, in cells with pWosA, *flaA* expression was maintained at high levels during the consolidation period (T_5 to T_6).

The disruption of the wild-type chromosomal copy of the *wosA* gene causes a slight decrease in swarming. To determine the role of the *wosA* gene product in swarming, a null allele was constructed by disrupting the wild-type chromosomal copy by homologous recombination with an internal fragment of *wosA*

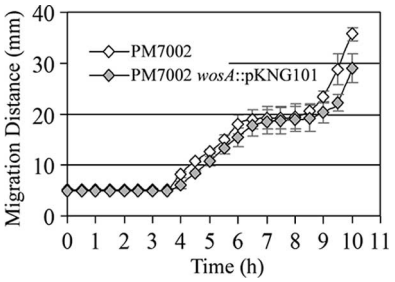

FIG. 4. Swarming migration of a *wosA* mutant. Wild-type *P. mirabilis* PM7002 and PM7002 carrying a chromosomal disruption of *wosA* (PM7002 *wosA*::pKNG101) were grown overnight with shaking at 37°C in LB or LB supplemented with streptomycin at 35 μg/ml, respectively. Cultures were adjusted to the same OD_{600}, 4 μl of each culture was spotted onto a 1.5% LB agar plate, and swarming diameters were measured every 30 min over a 10-h period. Values represent the means of results from four independent experiments. Error bars, SDs.

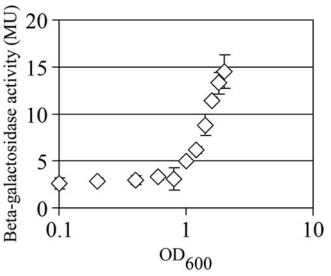

FIG. 5. Growth phase-dependent expression of *wosA* in liquid culture. Wild-type *P. mirabilis* PM7002 carrying a chromosomal insertion of a *wosA-lacZ* transcriptional fusion (PM7002 *wosA*::*wosA-lacZ*) was grown with shaking at 37°C in LB supplemented with streptomycin at 35 μg/ml. The culture was sampled in duplicate at various OD_{600}s and assayed for β-galactosidase activity. Values are the means of results for duplicate samples from three independent experiments. Error bars, SDs.

cloned into the suicide vector pKNG101 (25). The swarming migration of the wild-type PM7002 and that of the isogenic *wosA* null mutant are shown in Fig. 4. The PM7002 *wosA*::pKNG101 strain exhibited a mild but reproducible swarming defect relative to the swarming of the wild type. After 10 h of growth, the *wosA* mutant strain had migrated a significantly smaller distance than the wild-type PM7002 strain (ANOVA: $F_{1,6} = 19.02$; $P = 0.0047$). The PM7002 *wosA*::pKNG101 strain also exhibited a slight swimming defect in 0.3% soft agar, in which migration was decreased by 11% relative to that of the wild type (data not shown).

***wosA* gene expression is regulated in a growth phase-dependent manner.** To investigate the expression of *wosA*, β-galactosidase from a *wosA-lacZ* single-copy transcriptional fusion was measured during growth. This fusion was constructed at the native *wosA* locus and maintains a wild-type copy of *wosA*. Initial assays of samples plated to establish synchronously differentiated swarmer cells demonstrated relatively high levels of *wosA* expression at early time points after plating (data not shown). To determine if the activity observed was due to the expression of *wosA* in liquid culture, a low-density culture of the PM7002 *wosA*::*wosA-lacZ* strain was used to inoculate LB containing streptomycin and β-galactosidase activity from cells taken at various culture densities was determined. The results are shown in Fig. 5. The level of transcription of *wosA* remained low (~3 Miller units [MU]) until the culture reached an $OD_{600}$of 0.8, and it rapidly increased up to a value of 14.5 ± 1.8 MU in stationary phase. The resulting increase in transcription from early log phase to stationary phase was approximately 4.8-fold. However, no extracellular factors that had an effect on *wosA* expression were identified through the use of cell-free spent medium and the *wosA-lacZ* fusion strain (data not shown).

***wosA* gene expression during growth on plates.** To minimize carryover from the growth phase-dependent expression of *wosA* in the liquid inoculum, *P. mirabilis* strain PM7002 *wosA*::*wosA-lacZ* was plated onto several plates in parallel at a relatively low density (OD_{600} = 0.4) to produce synchronously differentiated cells. Cells taken at 1-h intervals after plating were assayed for β-galactosidase activity, and the results are shown in Fig. 6. At 1 h postplating (T_1), *wosA* expression was at its lowest level, with 3.01 ± 0.11 MU. β-galactosidase activity increased approximately 1.5-fold during hours 2 to 6 and reached 6.31 ± 0.72 MU by 8 h, resulting in a total increase of 2-fold. Therefore, despite considerable variation from one time point to the next, there was a significant linear increase in *wosA* expression over the entire course of the experiment ($\beta = 0.344$; $t_{25} = 6.76$; $P < 0.00001$). In addition, *wosA* expression is unlikely to be dependent on a feedback mechanism, as there was no significant difference in β-galactosidase activity from single-copy *wosA-lacZ* fusions in a wild-type background and in a *wosA*::Km^r background after 6 h of growth on plates (2.6 ± 0.1 and 2.5 ± 0.1 MU, respectively) or in liquid medium (4.3 ± 0.2 and 4.1 ± 0.03 MU, respectively) (data not shown).

***wosA* gene expression is independent of the putrescine signaling pathway.** The *wosA* gene was identified as a gene that when overexpressed restored the swarming phenotype to a mutant, PM437 (*speA*::mini-Tn*5lacZ1*), defective in the production of the extracellular signaling molecule putrescine and therefore delayed in swarmer cell differentiation and defective in swarming (43). The overexpression of the *wosA* gene was

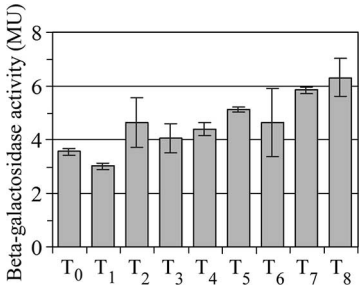

FIG. 6. Expression of *wosA* during the swarm cycle. Wild-type *P. mirabilis* PM7002 carrying a chromosomal insertion of a *wosA-lacZ* transcriptional fusion (PM7002 *wosA*::*wosA-lacZ*) was grown with shaking at 37°C in LB supplemented with streptomycin at 35 μg/ml. At an OD_{600} of 0.4, 1-ml samples of duplicate cultures were harvested for assaying β-galactosidase activity (T_0), and 200-μl aliquots of the cultures were plated onto several LB agar plates in parallel to produce synchronously differentiating cells. Plates were harvested in phosphate-buffered saline at 1-h intervals, the OD_{600} at each time point (T_1 to T_6) was determined, and cultures were assayed for β-galactosidase activity. Values are the means of results for duplicate samples from three independent experiments. Error bars, SDs.

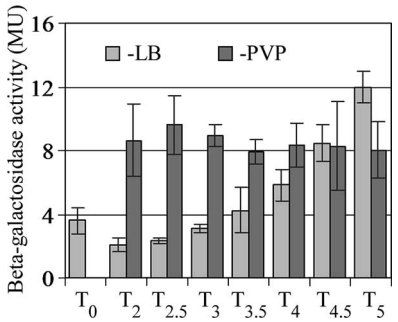

FIG. 7. Effect of increasing medium viscosity on *wosA* gene expression. Wild-type *P. mirabilis* PM7002 carrying a chromosomal insertion of a *wosA-lacZ* transcriptional fusion (PM7002 *wosA*::*wosA-lacZ*) was incubated with shaking at 37°C in LB medium and LB medium containing 15% PVP (wt/vol). From 2 h postinoculation (T_2), cells were harvested every 30 min and assayed for β-galactosidase activity. T_0 corresponds to *wosA-lacZ* expression in the inoculum. Values are the means of results for duplicate samples from three independent experiments. Error bars, SDs.

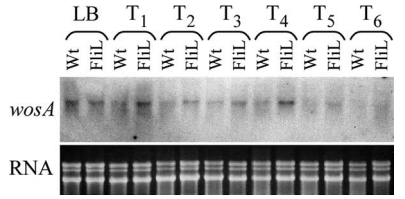

FIG. 8. Effect of a *fliL* mutation on *wosA* transcription. Northern hybridization of a *wosA* probe to total RNA from wild-type *P. mirabilis* BB2000 (Wt) and BB2204 (FliL) (top panel). Strain samples were taken from the liquid inoculum at an OD_{600} of 0.4 (LB) and from synchronous plate cultures at 1-h intervals over a swarmer cell differentiation cycle (T_1 to T_6). A representative ethidium bromide-stained gel containing one-fifth of the RNA used in the *wosA* Northern blot is shown in the bottom panel. Similar results were obtained from RNAs isolated from a second set of independently grown cells (data not shown).

also able to eliminate the defect in swarming in a *speB* mutant, PM439 (*speB*::Sm) (data not shown). To determine if putrescine is involved in regulating the expression of *wosA*, cells plated onto LB agar containing 50 μM putrescine were assayed for β-galactosidase activity as described in the previous paragraph. The addition of 50 μM putrescine was previously shown to eliminate the swarming defect of the PM437 (*speA*::mini-Tn*5lacZ1*) and PM439 (*speB*::Sm) mutants (43). No significant difference (ANOVA: $F_{1,2} = 4.73$; $P = 0.162$) in β-galactosidase activity in cells in the absence and those in the presence of 50 μM putrescine was observed after 7 h of growth on plates (5.85 ± 0.13 and 4.66 ± 0.79 MU, respectively). In addition, *wosA* overexpression did not alter the levels of the extracellular signal putrescine, as assayed by the ability of cell-free spent medium from PM7002 with the pBC vector and PM7002 with the pWosA plasmid to repress putrescine expression in the mutant PM437 (*speA*::mini-Tn*5lacZ1*) background. No significant difference (ANOVA: $F_{1,4} = 0.052$; $P = 0.831$) between these two strains was observed when the strains were assayed for putrescine expression (145.29 ± 27.24 and 140.91 ± 19.01 MU, respectively), indicating that WosA functions by bypassing the putrescine signaling pathway.

Increasing medium viscosity activates *wosA* transcription. In an attempt to better understand how WosA might be functioning, we assayed other *P. mirabilis* mutant strains known to induce swarmer cell differentiation under normally noninducing conditions. Since *wosA* overexpression leads to an increase in *flhDC* transcription, it was possible that WosA functions by decreasing RsbA or RcsC levels. The mutation of *rsbA* or *rcsC* in *P. mirabilis* leads to a precocious swarming phenotype (7). However, no difference in β-galactosidase activity between an *rsbA*::mini-Tn*5lacZ1* strain and an *rcsC*::mini-Tn*5lacZ1* strain carrying either the pBC vector or the pWosA plasmid was observed (data not shown).

Belas and Suvanasuthi (9) showed that conditions that inhibit flagellar rotation, such as high viscosity and the tethering of flagella, trigger swarmer cell differentiation in *P. mirabilis*. Since *wosA* overexpression leads to constitutive swarmer cell differentiation, we examined the effect of increasing the viscosity of the growth medium on *wosA* expression. The *P. mirabilis* strain PM7002 *wosA*::*wosA-lacZ* was grown in both LB and LB medium containing 15% PVP, and β-galactosidase activity from cells collected at specified time points was assayed. Cell numbers were too low at 1 h postinoculation for an accurate assay, so the first samples were taken at T_2. As described previously (9), vegetative cells continued to swim in LB medium and ceased swimming in LB medium containing 15% PVP. The expression of *wosA-lacZ* was upregulated approximately fourfold after the cells were exposed to LB–15% PVP compared to the expression during growth in LB (T_2 and $T_{2.5}$) (Fig. 7). Moreover, *wosA* expression remained elevated throughout the entire period of growth in LB–15% PVP. Swarmer cells were observed from T_3 to $T_{4.5}$ and were also unable to move in 15% PVP, despite the presence of flagella (data not shown). It should also be noted that the level of *wosA* expression is likely underestimated, as some cell loss was unavoidable when cells were pelleted from LB containing PVP. In LB medium alone, *wosA* expression increased with increasing cell density, as was shown above.

To determine whether the effect of medium viscosity on *wosA-lacZ* expression required the presence of flagella, expression in a *flaA*::Cm^r mutant that does not produce a flagellar filament was examined. In two independent experiments, the activation of *wosA-lacZ* by increased medium viscosity was reduced in a *flaA*::Cm^r background, relative to that in the wild-type background, by an average of 31% (2.5-fold versus 3.3-fold activation, respectively). These results indicate that although a flagellar filament is required for the optimal induction of *wosA* by increased viscosity, there is an additional pathway that contributes to *wosA* activation.

Belas and Suvanasuthi (9) also examined *P. mirabilis* swarming mutants with defects in their ability to sense and respond to surface signals. The *fliL* mutation resulted in an exaggerated swarmer cell elongation phenotype similar to the phenotype observed when *wosA* was overexpressed. One explanation for the similar phenotypes is that a mutation in *fliL* results in the upregulation of *wosA* expression. Therefore, we assayed *wosA* transcript levels in wild-type *P. mirabilis* BB2000 and strain BB2204 (*fliL*::Tn*5* Cm) by Northern blot analysis of RNA prepared from synchronously differentiating cells. The presence of the *fliL* mutation resulted in increased *wosA* transcript

accumulation at all time points (Fig. 8, top panel). In addition, we observed a slight increase in *flhDC* transcript levels in the *fliL* strain compared to those in the wild type at all time points (data not shown). This increase may be the result of increased transcription of *wosA*, as we know that increased *wosA* levels lead to increased *flhDC* levels (Fig. 3).

DISCUSSION

We have identified a *P. mirabilis* gene, *wosA*, that when overexpressed results in constitutive swarmer cell differentiation and a hyperswarming phenotype. The hyperswarming phenotype associated with multicopy *wosA* did not result from decreasing the lag in swarming initiation, as has been shown for precocious mutants with alterations in the RcsC/RcsD (RsbA)/RcsB signal transduction pathway (7). Nor is the swarming phenotype due to both decreasing the lag and increasing swarming velocity, as was reported previously for *flhDC* overexpression (16). Rather, the pWosA strain exhibits a hyperswarming phenotype by increasing swarming velocity while still maintaining temporal control of the onset of swarmer cell migration. Increasing the expression of WosA resulted in a corresponding increase in *flhDC* mRNA accumulation (Fig. 3). The increase in swarming velocity is therefore likely to be due to increased levels of FlhDC. The increased *flhDC* expression in the multicopy pWosA-containing strain at time points during the swarm cycle (Fig. 3) also likely accounts for the shorter consolidation period. It is not clear why this strain also exhibited increased swimming motility (data not shown). It has been reported that in *P. mirabilis* and other swarming bacteria, including *S. marcescens*, *Salmonella* serovar Typhimurium, and *E. coli*, swarmer cells show prolonged smooth swimming when suspended in liquid (7, 19). This reasoning may explain the increase in swimming velocity; however, we cannot rule out an increase in flagellar rotation as the cause for the increased swimming velocity observed.

Despite the increased FlhDC expression and constitutive swarmer cell differentiation resulting from increased WosA expression, swarming in the WosA-overexpressing strain was initiated at the same time as that in the wild-type strain. In *P. mirabilis* strains that specifically overexpressed FlhDC, swarming was initiated earlier than that in wild-type strains; however, it was not accompanied by constitutive swarmer cell differentiation. Therefore, while the WosA-overexpressing strain was insensitive to differentiation controls under noninducing conditions, it still responded appropriately on solid surfaces to initiate swarming. This result provides evidence that in addition to the signals required to initiate differentiation, a signal is required for the initiation of swarming. In *S. liquefaciens*, two separate regulatory systems are proposed to control swarming (17). Swarmer cell differentiation is controlled by the *flhDC* operon, while swarming migration is controlled via the production of a biosurfactant that is facilitated by the SwrI-dependent autoinducer. It is possible that the WosA-overexpressing strain acts in a similar manner and requires the accumulation of a biosurfactant, such as colony migration factor (Cmf) (18), before swarming can be initiated. Although the *wosA* gene was identified by its ability at high copy numbers to suppress a mutation in the putrescine signaling pathway, our data indicate that WosA acts independently of this pathway to increase swarming migration.

In *P. mirabilis*, the inhibition of flagellar rotation is one requirement for swarmer cell differentiation (1, 15, 37). Medium conditions that restrict flagellar rotation, including the addition of anti-flagellar antiserum to the growth medium or an increase in the medium viscosity, result in the induction of swarmer cell differentiation in normally noninducing liquid medium (9). Mutations in the *fliL*, *fliG*, and *fliF* genes that encode the components of the flagellar basal body also result in mutant strains that produce abnormally long swarmer cells or produce swarmer cells under noninducing conditions, similar to the mutant phenotype observed for the *Caulobacter crescentus fliL* mutant strain (9). These mutations associated with the flagellar basal body have been proposed to cause defects in surface sensing. Moreover, the *fliL* mutation was shown to upregulate two known virulence genes of *P. mirabilis*, *zapA* and *hpmB*. These genes encode a major extracellular zinc metalloprotease and a hemolysin, respectively, and are known to be upregulated only in the differentiated swarmer cell (5), implying a role for FliL in the regulation of virulence genes (9).

Although the *wosA* gene was isolated based on the swarming phenotypes associated with overexpression, our studies suggest that *wosA* may be important in the process of swarmer cell differentiation. First, the expression of *wosA* increases during swarmer cell differentiation (Fig. 6). Second, the overexpression of *wosA* leads to increased *flhDC* expression, implying that WosA either directly or indirectly regulates *flhDC* expression and leads to swarmer cell differentiation in noninducing liquid medium. Finally, growth in high-viscosity medium (15% PVP) or a mutation of the *fliL* gene increased *wosA* expression (Fig. 7 and 8). These conditions are predicted to inhibit flagellar rotation or function, and both result in swarmer cell differentiation (1, 15, 37). Bioinformatic analyses of the deduced primary sequence of the WosA protein suggest that the protein contains a single transmembrane domain at the N-terminal end and give a high probability that the protein would be oriented in the out-to-in orientation. As no DNA binding domain has been identified, it is likely that *wosA* indirectly regulates *flhDC* expression. However, if FliL is found in the flagellar basal body, as hypothesized by Belas and Suvanasuthi (9), the putative location and orientation of the WosA protein may potentially allow an interaction between WosA and FliL. Although speculative, our data suggest that WosA may be involved in a signal transduction pathway that senses solid surfaces, possibly via interacting with FliL either directly or indirectly, and couple this to increased FlhDC expression.

The use of a *flaA* mutant demonstrated that although an intact flagellum is needed for the full induction of *wosA-lacZ* in high-viscosity media, a flagellum-independent pathway for *wosA* activation also exists under these conditions. There are at least two possibilities for this second pathway. First, although we could find no evidence for a role of extracellular signals in *wosA* expression, a highly unstable, *wosA*-activating signal may diffuse away from cells more slowly in viscous media. Second, viscous media may also elicit cell surface changes that are independent of the inhibition of flagellar rotation and trigger a second surface-sensing mechanism. Unpublished studies from our lab have uncovered evidence for this second mechanism, where mutants defective in O-antigen are unable to differen-

tiate on solid surfaces yet swim normally in liquid, indicating that flagellar function is not impaired. Therefore, highly viscous media may also activate *wosA* via cell surface changes sensed by O-antigen, and experiments are in progress to test this possibility.

A null allele of *wosA* resulted in a slight decrease in swarming motility (Fig. 4). While this effect was subtle, it may reflect the presence of more than one protein that functions in a manner similar to that of WosA. A common theme in bacterial differentiation is the presence of multiple checkpoints that control each step leading to a differentiated cell. In *P. mirabilis*, the process of swarmer cell differentiation is controlled by multiple signals and regulatory checkpoints, many of which act through the *flhDC* flagellar master operon that controls the flagellar gene hierarchy and swarmer cell differentiation. WosA may act in one of these checkpoint pathways. In summary, although bioinformatics analyses do not provide many clues regarding the actual function of WosA, this study indicates that it regulates FlhDC expression, possibly through the sensing of environmental signals that result when cells encounter a solid surface.

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REFERENCES

1. **Alavi, M., and R. Belas.** 2001. Surface sensing, swarmer cell differentiation, and biofilm development. Methods Enzymol. **336:**29–40.
2. **Alberti, L., and R. M. Harshey.** 1990. Differentiation of *Serratia marcescens* 274 into swimmer and swarmer cells. J. Bacteriol. **172:**4322–4328.
3. **Allison, C., and C. Hughes.** 1991. Bacterial swarming: an example of prokaryotic differentiation and multicellular behaviour. Sci. Prog. **75:**403–422.
4. **Allison, C., H. C. Lai, D. Gygi, and C. Hughes.** 1993. Cell differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemoattractant for swarming cells. Mol. Microbiol. **8:**53–60.
5. **Allison, C., H. C. Lai, and C. Hughes.** 1992. Co-ordinate expression of virulence genes during swarm-cell differentiation and population migration of *Proteus mirabilis*. Mol. Microbiol. **6:**1583–1591.
6. **Belas, R., D. Erskine, and D. Flaherty.** 1991. Transposon mutagenesis in *Proteus mirabilis*. J. Bacteriol. **173:**6289–6293.
7. **Belas, R., R. Schneider, and M. Melch.** 1998. Characterization of *Proteus mirabilis* precocious swarming mutants: identification of *rsbA*, encoding a regulator of swarming behavior. J. Bacteriol. **180:**6126–6139.
8. **Belas, R., M. Simon, and M. Silverman.** 1986. Regulation of lateral flagella gene transcription in *Vibrio parahaemolyticus*. J. Bacteriol. **167:**210–218.
9. **Belas, R., and R. Suvanasuthi.** 2005. The ability of *Proteus mirabilis* to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein. J. Bacteriol. **187:**6789–6803.
10. **Chilcott, G. S., and K. T. Hughes.** 2000. Coupling of flagellar gene expression to flagellar assembly in *Salmonella enterica* serovar Typhimurium and *Escherichia coli*. Microbiol. Mol. Biol. Rev. **64:**694–708.
11. **Dufour, A., R. B. Furness, and C. Hughes.** 1998. Novel genes that upregulate the *Proteus mirabilis flhDC* master operon controlling flagellar biogenesis and swarming. Mol. Microbiol. **29:**741–751.
12. **Eberl, L., G. Christiansen, S. Molin, and M. Givskov.** 1996. Differentiation of *Serratia liquefaciens* into swarm cells is controlled by the expression of the *flhD* master operon. J. Bacteriol. **178:**554–559.
13. **Farinha, M. A., and A. M. Kropinski.** 1990. Construction of broad-host-range plasmid vectors for easy visible selection and analysis of promoters. J. Bacteriol. **172:**3496–3499.
14. **Francez-Charlot, A., B. Laugel, A. Van Gemert, N. Dubarry, F. Wiorowski, M. P. Castanie-Cornet, C. Gutierrez, and K. Cam.** 2003. RcsCDB His-Asp phosphorelay system negatively regulates the *flhDC* operon in *Escherichia coli*. Mol. Microbiol. **49:**823–832.
15. **Fraser, G. M., and C. Hughes.** 1999. Swarming motility. Curr. Opin. Microbiol. **2:**630–635.
16. **Furness, R. B., G. M. Fraser, N. A. Hay, and C. Hughes.** 1997. Negative feedback from a *Proteus* class II flagellum export defect to the *flhDC* master operon controlling cell division and flagellum assembly. J. Bacteriol. **179:**5585–5588.
17. **Givskov, M., J. Ostling, L. Eberl, P. W. Lindum, A. B. Christensen, G. Christiansen, S. Molin, and S. Kjelleberg.** 1998. Two separate regulatory systems participate in control of swarming motility of *Serratia liquefaciens* MG1. J. Bacteriol. **180:**742–745.
18. **Gygi, D., M. M. Rahman, H. C. Lai, R. Carlson, J. Guard-Petter, and C. Hughes.** 1995. A cell-surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. Mol. Microbiol. **17:**1167–1175.
19. **Harshey, R. M.** 2003. Bacterial motility on a surface: many ways to a common goal. Annu. Rev. Microbiol. **57:**249–273.
20. **Harshey, R. M., and T. Matsuyama.** 1994. Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. Proc. Natl. Acad. Sci. USA **91:**8631–8635.
21. **Hay, N. A., D. J. Tipper, D. Gygi, and C. Hughes.** 1997. A nonswarming mutant of *Proteus mirabilis* lacks the Lrp global transcriptional regulator. J. Bacteriol. **179:**4741–4746.
22. **Henrichsen, J.** 1972. Bacterial surface translocation: a survey and a classification. Bacteriol. Rev. **36:**478–503.
23. **Hofmann, K., and W. Stoffel.** 1993. TMbase—a database of membrane spanning protein segments. Biol. Chem. Hoppe-Seyler **374:**166.
24. **Jeong, H., J. H. Yim, C. Lee, S. H. Choi, Y. K. Park, S. H. Yoon, C. G. Hur, H. Y. Kang, D. Kim, H. H. Lee, K. H. Park, S. H. Park, H. S. Park, H. K. Lee, T. K. Oh, and J. F. Kim.** 2005. Genomic blueprint of *Hahella chejuensis*, a marine microbe producing an algicidal agent. Nucleic Acids Res. **33:**7066–7073.
25. **Kaniga, K., I. Delor, and G. R. Cornelis.** 1991. A wide-host-range suicide vector for improving reverse genetics in gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. Gene **109:**137–141.
26. **Kawagishi, I., M. Imagawa, Y. Imae, L. McCarter, and M. Homma.** 1996. The sodium-driven polar flagellar motor of marine *Vibrio* as the mechanosensor that regulates lateral flagellar expression. Mol. Microbiol. **20:**693–699.
27. **Liaw, S. J., H. C. Lai, S. W. Ho, K. T. Luh, and W. B. Wang.** 2001. Characterisation of p-nitrophenylglycerol-resistant *Proteus mirabilis* super-swarming mutants. J. Med. Microbiol. **50:**1039–1048.
28. **Majdalani, N., and S. Gottesman.** 2005. The Rcs phosphorelay: a complex signal transduction system. Annu. Rev. Microbiol. **59:**379–405.
29. **Marchler-Bauer, A., and S. H. Bryant.** 2004. CD-Search: protein domain annotations on the fly. Nucleic Acids Res. **32:**W327–W331.
30. **McCarter, L., M. Hilmen, and M. Silverman.** 1988. Flagellar dynamometer controls swarmer cell differentiation of *V. parahaemolyticus*. Cell **54:**345–351.
31. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
32. **Miller, V. L., and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. J. Bacteriol. **170:**2575–2583.
33. **Nishimura, A., and Y. Hirota.** 1989. A cell division regulatory mechanism controls the flagellar regulon in *Escherichia coli*. Mol. Gen. Genet. **216:**340–346.
34. **Ott, L.** 1988. An introduction to statistical methods and data analysis, 3rd ed. PWS-Kent Publishing Co., Boston, MA.
35. **Pruss, B. M., and P. Matsumura.** 1996. A regulator of the flagellar regulon of *Escherichia coli*, *flhD*, also affects cell division. J. Bacteriol. **178:**668–674.
36. **Rather, P. N.** 2005. Swarmer cell differentiation in *Proteus mirabilis*. Environ. Microbiol. **7:**1065–1073.
37. **Rauprich, O., M. Matsushita, C. J. Weijer, F. Siegert, S. E. Esipov, and J. A. Shapiro.** 1996. Periodic phenomena in *Proteus mirabilis* swarm colony development. J. Bacteriol. **178:**6525–6538.
38. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
39. **Shi, W., Y. Zhou, J. Wild, J. Adler, and C. A. Gross.** 1992. DnaK, DnaJ, and GrpE are required for flagellum synthesis in *Escherichia coli*. J. Bacteriol. **174:**6256–6263.
40. **Shin, S., and C. Park.** 1995. Modulation of flagellar expression in *Escherichia coli* by acetyl phosphate and the osmoregulator OmpR. J. Bacteriol. **177:**4696–4702.
41. **Silverman, M., and M. Simon.** 1974. Characterization of *Escherichia coli* flagellar mutants that are insensitive to catabolite repression. J. Bacteriol. **120:**1196–1203.
42. **Stevenson, L. G., and P. N. Rather.** 2006. A novel gene involved in

regulating the flagellar gene cascade in *Proteus mirabilis*. J. Bacteriol. **188:**7830–7839.
43. **Sturgill, G., and P. N. Rather.** 2004. Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. Mol. Microbiol. **51:**437–446.
44. **Takeda, S., Y. Fujisawa, M. Matsubara, H. Aiba, and T. Mizuno.** 2001. A novel feature of the multistep phosphorelay in *Escherichia coli*: a revised model of the RcsC → YojN → RcsB signalling pathway implicated in capsular synthesis and swarming behaviour. Mol. Microbiol. **40:**440–450.
45. **Ulitzur, S.** 1974. Induction of swarming in *Vibrio parahaemolyticus*. Arch. Microbiol. **101:**357–363.
46. **Yokota, T., and J. S. Gots.** 1970. Requirement of adenosine 3′,5′-cyclic phosphate for flagella formation in *Escherichia coli* and *Salmonella typhimurium*. J. Bacteriol. **103:**513–516.
47. **Young, G. M., M. J. Smith, S. A. Minnich, and V. L. Miller.** 1999. The *Yersinia enterocolitica* motility master regulatory operon, *flhDC*, is required for flagellin production, swimming motility, and swarming motility. J. Bacteriol. **181:**2823–2833.