Chemotactic Control of the Two Flagellar Systems of *Rhodobacter sphaeroides* Is Mediated by Different Sets of CheY and FlhM Proteins^{▽†}

Ana Martínez del Campo,¹ Teresa Ballado,¹ Javier de la Mora,¹ Sebastian Poggio,²
Laura Camarena,² and Georges Dreyfus^{1*}

Instituto de Fisiología Celular¹ and Instituto de Investigaciones Biomédicas,² Universidad Nacional Autónoma de México, México 04510, México

Received 9 May 2007/Accepted 28 August 2007

***Rhodobacter sphaeroides* expresses two different flagellar systems, a subpolar flagellum (fla1) and multiple polar flagella (fla2). These structures are encoded by different sets of flagellar genes. The chemotactic control of the subpolar flagellum (fla1) is mediated by three of the six different CheY proteins (CheY6, CheY4, or CheY3). We show evidence that CheY1, CheY2, and CheY5 control the chemotactic behavior mediated by fla2 flagella and that RSP6099 encodes the fla2 FlhM protein.**

Many bacteria move by using flagella as the locomotive organelle. Flagella alternate between clockwise and counter-clockwise rotation or in some cases between rotation and brief stop periods, which allow the bacterial cell to swim in a linear trajectory or to reorient (1, 14, 15). Bacterial taxis is achieved by modifying the frequency of reorientation events. The bias in the frequency of reorientation is modified by the interaction of the phosphorylated form of the response regulator CheY-P with the switch protein (FlhM), which is part of the motor-switch structure located at the base of the flagellum. The concentration of CheY-P is regulated by the chemotactic system (3).

A limited number of bacteria possess dual flagellar systems and are able to express two different flagellum types: a polar flagellum for swimming and lateral flagella for swarming; these microorganisms include *Vibrio parahaemolyticus* (27), *Vibrio alginolyticus* (8), *Aeromonas* spp. (26), *Azospirillum brasilense* (18), *Rhodospirillum centenum* (17), *Helicobacter mustelae* (19), and *Plesiomonas shigelloides* (7). The best-characterized examples are those that belong to the *Vibrio* genus. The ability to synthesize two different types of flagella allows these microorganisms to populate various niches (16).

Rhodobacter sphaeroides is a facultative nonsulfur photosynthetic bacterium. Its genome sequence revealed many intriguing features related to motility and chemotaxis (13), including the presence of two flagellar gene sets. The genes that belong to the first set (fla1) are expressed constitutively and allow the strong swimming previously reported for the WS8 wild-type strain (1, 10, 21, 22). The genes of the second flagellar set are not expressed in the wild-type WS8 strain; however, strains that express it can be isolated. In contrast with the case for

other dual-flagellum systems, the fla2 genes of *R. sphaeroides* produce polar flagella that allow swimming (21).

Several chemotactic genes are also reiterated (two *cheB* genes, three *cheR* genes, four *cheA* and *cheW* genes, and six *cheY* genes). In spite of this, only some of these gene copies are required when the cell is swimming with the fla1 flagellum. For example, it has been reported that only CheY6 and either CheY3 or CheY4 are required for chemotaxis mediated by the fla1 flagellum (23), despite the fact that the six *cheY* genes are expressed (6). The presence of a second functional flagellum in *R. sphaeroides* suggests that some of the chemotactic genes could be involved in its tactic control. To test this hypothesis, we investigated the possible role of the six CheY proteins in swimming mediated by fla2 flagella.

Bacterial strains, plasmids, and oligonucleotides used in this work are listed in Table 1. *R. sphaeroides* cell cultures were grown in liquid or solid Siström's minimal medium (29) as described previously (21). Recombinant DNA techniques were carried out by standard procedures (2).

Isolation of a fla2⁺ strain. To test if swimming mediated by fla2 flagella could be influenced by any of the CheY proteins, we isolated a strain showing the fla2⁺ phenotype from the SP13 strain (*fleQΔ::Kan*), which lacks the master regulatory protein FleQ (22), following a procedure described recently (21). Swimming cells isolated from this strain represent those that express the fla2 flagella. The resultant strain, AM1, showed a swimming pattern similar to that of the SP18 fla2⁺ strain, which has been previously described (21) (see Fig. S1 in the supplemental material). To confirm the fla2⁺ phenotype, expression of the FlgE2 protein in the AM1 strain was detected by Western blotting (see Fig. S2 in the supplemental material). Since motility is not easily quantifiable in the stab cultures, we decided to test if swimming dependent on the fla2 flagellum could also be observed in swimming plates. As shown in Fig. 1, the fla2⁺-dependent motility of the AM1 strain was also observed in this assay. Therefore, all further motility assays were performed using swimming plates. From these experiments we noticed differences in the patterns of attractant preference of the WS8 and AM1 strains. While, as previously reported (24), the WS8 strain produces increasingly larger swarms in succi-

* Corresponding author. Mailing address: Departamento de Genética Molecular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apdo. Postal 70-243 Cd. Universitaria, México DF 04510, México. Phone: (5255)5622-5618. Fax: (5255)5622-5611. E-mail: gdreyfus@ifc.unam.mx.

† Supplemental material for this article may be found at <http://jb.asm.org/>.

[▽] Published ahead of print on 21 September 2007.

TABLE 1. Bacterial strains, plasmids, and oligonucleotides used in this study

Strain, plasmid, or oligonucleotide	Relevant characteristic(s)	Source or reference
Strains		
<i>R. sphaeroides</i>		
WS8-N	Wild type; spontaneous Nal ^r	30
SP18	WS8 derivative; <i>flgC</i> ::Kan	21
SS2	WS8 derivative; <i>flgC</i> ::Kan <i>flhA2</i> :: <i>uidA-aadA</i>	21
SP13	WS8 derivative; <i>fleQΔ</i> ::Kan	22
AM1	SP13 derivative <i>fla-2</i> ⁺	This work
RS2Y1	AM1 derivative; <i>cheY1</i> :: <i>aadA</i>	This work
RS2Y2	AM1 derivative; <i>cheY2</i> :: <i>aadA</i>	This work
RS2Y3	AM1 derivative; <i>cheY3</i> :: <i>aadA</i>	This work
RS2Y4	AM1 derivative; <i>cheY4Δ</i> :: <i>aadA</i>	This work
RS2Y5	AM1 derivative; <i>cheY5</i> :: <i>aadA</i>	This work
RS2Y6	AM1 derivative; <i>cheY6Δ</i> :: <i>aadA</i>	This work
AM2	AM1 derivative; <i>fliM2</i> :: <i>aadA</i>	This work
<i>E. coli</i>		
JM103	<i>hsdR4 Δ(lac-pro) F' traD36 proAB lacI^q ΔM15</i>	2
TOP10	Cloning strain	Invitrogen
S17-1	<i>recA endA thi hsdR RP4-2 Tc::Mu::Tn7; Tp^r Sm^r</i>	28
Plasmids		
pTZ19R	Cloning vector; pUC derivative; Ap ^r	Pharmacia
pJQ200mp18	Suicide vector used for gene replacement; Gm ^r	25
pRK415	pRK404 derivative; Tc ^r	9
pRKfliM2	pRK415 carrying <i>fliM2</i> ⁺	This work
pRKcheY1	pRK415 carrying <i>cheY1</i> ⁺	This work
pRKcheY2	pRK415 carrying <i>cheY2</i> ⁺	This work
pRKcheY5	pRK415 carrying <i>cheY5</i> ⁺	This work
Oligonucleotides		
<i>cheY1</i> fw	5'-CTAGTCTAGAGCGGAAGGTCACCAATTCAC-3'	This work
<i>cheY1</i> rev	5'-CCGGAATTTCGCGTATCCTCGATACGATCG-3'	This work
<i>cheY2</i> fw	5'-CTAGTCTAGACGAAACTGTTGCGATGACATC-3'	This work
<i>cheY2</i> rev	5'-CCGGAATTCCCCACGGCCTCCTCGACGATCTC-3'	This work
<i>cheY3</i> fw	5'-CTAGTCTAGACGGCGCCACGGGGCGGCGGC-3'	This work
<i>cheY3</i> rev	5'-CCGGAATTCCGATCTTCCGGCGAGTCGCTG-3'	This work
<i>cheY4</i> fw1	5'-CGCGGATCCGCCCCAGGATGTGCTGCGCG-3'	This work
<i>cheY4</i> rev2	5'-CCGGAATTCGAGGACGGTTTTCGTCATGGC-3'	This work
<i>heY4</i> fw3	5'-CGGGAATTCAAGAAGCTTCTTGGCTGA-3'	This work
<i>cheY4</i> rev4	5'-TTTTCTGCAGCGTATTCGGCCAGCGCCGCC-3'	This work
<i>cheY5</i> fw1	5'-CTAGTCTAGACGCCCATGGAGACGGCGATG-3'	This work
<i>cheY5</i> rev2	5'-CCGGAATTCGGACAGTCTCATCGCTACAG-3'	This work
<i>cheY6</i> fw1	5'-CTAGTCTAGAATCTCGCGCGCCTCGGACGAG-3'	This work
<i>cheY6</i> rev2	5'-CCGGAATTCGACATTGTAGGGCATCGG-3'	This work
<i>cheY6</i> fw3	5'-CCGGAATTCGGACGCTGATGGCCGCTGA-3'	This work
<i>cheY6</i> rev4	5'-CTAGTCTAGACTCCGTCGCGGCGGCCCTC-3'	This work
Spc fw	5'-CCTGGAATTCGGGCAGATCCGTG-3'	This work
Spc rv	5'-TCATGAATTCTCTCCCAATTTGTG-3'	This work
<i>fliM2</i> fw	5'-CGCGGATCCTCTTCGAGAAGTTGCGCCGCGC-3'	This work
<i>fliM2</i> rv	5'-TGCTCTAGACGCCACGAGATCGACCCGCTC-3'	This work
<i>aadA</i> fw	5'-CCGAGCTCCCTGAAGCCAGGGCAGATCCGTG-3'	This work
<i>aadA</i> rv	5'-CCGAGCTCTTCATGATATATCTCCAATTTG-3'	This work

nate, acetate, and propionate (17.4 ± 2.9 , 23.7 ± 3.5 , and 27.7 ± 3.8 mm, respectively), the AM1 strain shows no detectable preference for any of the attractants tested (23.4 ± 1.1 mm) (Fig. 1A).

Swimming behavior of the *cheY* mutants. To determine the role of the six *CheY* proteins in *fla2*-dependent swimming, we proceeded to individually mutate each *cheY* gene by inverse PCR using the oligonucleotides described in Table 1, followed by the insertion of a nonpolar *Spc^r* cassette (22). The mutant alleles were subcloned into the suicide plasmid pJQ200mp18 (25). The resultant plasmids were then mobilized into *R. spha-*

eroides by conjugation according to previously reported procedures (5). Replacement of each of the *cheY* alleles was done for the WS8 wild-type and AM1 strains. The *Spc^r* Gm^s transconjugants were selected, and the allelic exchange was confirmed by PCR. The expression and assembly of the *fla2* or *fla1* flagellum was verified for all the *cheY* mutants obtained from the AM1 or WS8 strain by detection of the FlgE2 protein and by observation of assembled filaments with an electron microscope (see Fig. S2 in the supplemental material; also data not shown).

Analysis of the swimming phenotype produced by these mu-

FIG. 1. Soft agar motility assays of different strains. (A) Effects of different chemoattractants on the swimming motilities of WS8 (*fla1*⁺-dependent motility) and AM1 (*fla2*⁺-dependent motility). Plates containing Sistrom's minimal medium without succinate and 0.25% agar were supplemented with 100 μ M of the indicated attractant. The nonmotile SP13 strain was included as a negative control. (B) Swimming pattern of the AM1 strain carrying the indicated *cheY* mutation. In this experiment, succinate was added as a chemoattractant at a concentration of 100 μ M.

tant alleles in the wild-type WS8 genetic background revealed a clear reduction only in the swimming ring of the strain lacking *cheY6* (data not shown). This result is in agreement with previous reports showing that the chemotactic response of the *fla1* flagellum is controlled by CheY6 and CheY3 or CheY4 (23).

A different result was obtained when motility dependent on the *fla2* system was analyzed for each of the *cheY* mutants; in this case, mutants lacking *cheY1*, *cheY2*, or *cheY5* showed smaller swimming rings, suggesting a role of these genes in the chemotactic control of the *fla2* flagellum (Fig. 1B). This is the first time that a phenotype has been associated with the absence of CheY1, CheY2, and CheY5 or any other protein

encoded by chemotaxis operon 1. Complementation experiments with each individual mutant with its respective wild-type allele (see Fig. S5 in the supplemental material) confirm that *fla2*⁺ motility is controlled by the above-mentioned response regulators. Therefore, we propose that the CheY proteins encoded in operon 1 are involved in the chemotactic response mediated by *fla2*.

In addition, the effect of removing each of these response regulators in the AM1 strain was analyzed by challenging these mutants with three different attractants. For these assays, a 2- μ l aliquot of a stationary-phase culture was placed in quadruplicate on the surface of soft-agar (0.25%) swimming plates containing Sistrom's medium devoid of succinate and supplemented with 100 μ M of the attractant being tested (propionate, succinate, or acetate). After 48 h of incubation under aerobiosis at 30°C, swarm diameters were measured and compared (Fig. 2; also shown are representative images of swimming rings of the six *cheY* mutants in the presence of the three attractants). Our results show that the absence of CheY1, -2, or -5 renders the AM1 cells deficient in the chemotactic response to the three attractants tested, whereas cells lacking CheY3, CheY4, and CheY6 respond normally to these attractants, as does the control AM1 strain.

From our results, it can be concluded that the multiple CheY proteins of *R. sphaeroides* function in two independent groups. Chemotaxis of the *fla1* flagellum is controlled by CheY6 and either CheY3 or CheY4 (23), while the *fla2* flagellum is controlled by CheY1, CheY2, and CheY5. In addition, the different attractant preference patterns of the WS8 and AM1 strains suggest that these two functionally independent groups of CheY proteins are phosphorylated differentially. This could occur through differences in the affinities of the two sets of CheY proteins for the CheA proteins associated with the chemotactic receptors or through a complete separation of the two chemotactic pathways.

Identification of the *fliM2* gene. Although most of the genes belonging to the *fla2* system have been previously identified (21), *fliM2* has not been described. A BLAST search against the genome of *R. sphaeroides* 2.4.1 using the *fliM1* gene sequence as a query revealed the presence of an open reading frame (RSP6099) with low similarity, restricted to the C-terminal half of the hypothetical protein. The gene encoding this putative FliM protein appears to be in a monocistronic transcriptional unit (Fig. 3A). A mutant strain of RSP6099 (AM2 strain) is nonmotile in either stab tubes or swimming plates (Fig. 3B). Motility dependent on the *fla2* flagellum of this strain can be restored by complementation with a plasmid expressing the wild-type gene (Fig. 3B). Observation of negatively stained preparations of the AM2 strain revealed the presence of flagella, indicating that this gene is not absolutely indispensable for flagellar biosynthesis (data not shown). Although FliM is essential for flagellar biosynthesis in *Escherichia coli* (31), in some species a *fliM* mutant is still able to assemble filaments with a lower efficiency than the wild-type strain (32). A domain analysis of the protein sequence encoded by RSP6099 using the program CD-Search revealed a truncated FliM domain (see Fig. S3 in the supplemental material). This was due to the low similarity of the N terminus of RSP6099 to those of the FliM proteins that compose the FliM cluster of orthologous genes (COG1868) (see Fig. S3 in the

FIG. 2. Diameter of the swimming ring produced by AM1 and the different *cheY* mutants (AM1 derivatives). Diameters are the mean value from three independent experiments ($n = 12$) $\pm$ standard errors. Significance was assessed by one-way analysis of variance; *, $P < 0.001$. Representative images of the swimming rings produced by these strains are shown in the lower panel.

supplemental material). In particular, the conserved amino acids involved in CheY binding (4, 12, 20) are absent in RSP6099 (Fig. 3C), suggesting that this protein interacts with its cognate CheY proteins through a different signature. Interestingly, a search for other, similar putative FliM proteins revealed several candidates that also lack the amino acids relevant for CheY binding (see Fig. S4 in the supplemental material). These hypothetical proteins are found in other al-

FIG. 3. Genetic context, functional assay, and analysis of the N terminus of FliM2. (A) Genetic context of *fliM2* (RSP6099). Arrows indicate the direction of transcription. (B) Swimming assay and complementation analysis of the *fliM2* mutant: 1, AM1; 2, AM2 (*fliM2::aadA*); 3, AM2/pRK*fliM2*+. (C) Alignment of the N termini of several FliM proteins. *Silicibacter pomeroyi* (Spom), *R. sphaeroides* fla2 (Rsph2), *R. sphaeroides* fla1 (Rsph1), *Helicobacter pylori* (Hpyl), *Caulobacter crescentus* (Ccres), *Aeromonas hydrophila* (Ahyd), *Bordetella pertussis* (Bper), and *Escherichia coli* (Ecoli) protein sequences are shown.

phaproteobacteria, mainly those belonging to the *Roseobacter* family. By the same token, it has been reported that a FlIM homologue lacking the conventional CheY-binding motif was found in *Vibrio alginolyticus* (11). In this work we show that the two sets of flagella in *R. sphaeroides* are controlled by independent sets of CheY response regulators that interact with FlIM proteins that possibly belong to two different families.

We thank Aurora Osorio for helpful technical assistance and the IFC Molecular Biology Unit for sequencing facilities.

This work was supported by the Consejo Nacional de Ciencia y Tecnología, grants 47172/A-1 and P42600-Q.

REFERENCES

- Armitage, J. P., and R. M. Macnab. 1987. Unidirectional, intermittent rotation of the flagellum of *Rhodobacter sphaeroides*. *J. Bacteriol.* **169**:514–518.
- Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York, NY.
- Berg, H. C. 2003. The rotary motor of bacterial flagella. *Annu. Rev. Biochem.* **72**:19–54.
- Bren, A., and M. Eisenbach. 1998. The N terminus of the flagellar switch protein, FlIM, is the binding domain for the chemotactic response regulator, CheY. *J. Mol. Biol.* **278**:507–514.
- Davis, J., T. J. Donohue, and S. Kaplan. 1988. Construction, characterization, and complementation of a P_{uf}^{-} mutant of *Rhodobacter sphaeroides*. *J. Bacteriol.* **170**:320–329.
- Ferré, A., J. de la Mora, T. Ballado, L. Camarena, and G. Dreyfus. 2004. Biochemical study of multiple CheY response regulators of the chemotactic pathway of *Rhodobacter sphaeroides*. *J. Bacteriol.* **186**:5172–5177.
- Inoue, K., Y. Kosako, K. Suzuki, and T. Shimada. 1991. Peritrichous flagellation in *Plesiomonas shigelloides* strains. *Jpn. J. Med. Sci. Biol.* **44**:141–146.
- Kawagishi, I., Y. Maekawa, T. Atsumi, M. Homma, and Y. Imae. 1995. Isolation of the polar and lateral flagellum-defective mutants in *Vibrio alginolyticus* and identification of their flagellar driving energy sources. *J. Bacteriol.* **177**:5158–5160.
- Keen, N. T., S. Tamaki, D. Kobayashi, and D. Trollinger. 1988. Improved broad-host-range plasmids for DNA cloning in Gram-negative bacteria. *Gene* **70**:191–197.
- Kobayashi, K., T. Saitoh, D. S. Shah, K. Ohnishi, I. G. Goodfellow, R. E. Sockett, and S. I. Aizawa. 2003. Purification and characterization of the flagellar basal body of *Rhodobacter sphaeroides*. *J. Bacteriol.* **185**:5295–5300.
- Kojima, M., R. Kubo, T. Yakushi, M. Homma, and I. Kawagishi. 2007. The bidirectional polar and unidirectional lateral flagellar motors of *Vibrio alginolyticus* are controlled by a single CheY species. *Mol. Microbiol.* **64**:57–67.
- Lee, S. Y., H. S. Cho, J. G. Pelton, D. Yan, R. K. Henderson, D. S. King, L. Huang, S. Kustu, E. A. Berry, and D. E. Wemmer. 2001. Crystal structure of an activated response regulator bound to its target. *Nat. Struct. Biol.* **8**:52–56.
- Mackenzie, C., M. Choudhary, F. W. Larimer, P. F. Predki, S. Stilwagen, J. P. Armitage, R. D. Barber, T. J. Donohue, J. P. Hosler, J. E. Newman, J. P. Shapleigh, R. E. Sockett, J. Zeilstra-Ryalls, and S. Kaplan. 2001. The home stretch, a first analysis of the nearly completed genome of *Rhodobacter sphaeroides* 2.4.1. *Photosynth. Res.* **70**:19–41.
- Macnab, R. M. 1996. Flagella and motility, p. 123–145. In F. C. Niedhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli and Salmonella: cellular and molecular biology*. American Society for Microbiology, Washington, DC.
- Macnab, R. M. 2003. How bacteria assemble flagella. *Annu. Rev. Microbiol.* **57**:77–100.
- McCarter, L. L. 2006. Motility and chemotaxis, p. 115–132. In F. L. Thompson, B. Austin, and J. Swings (ed.), *The biology of vibrios*. American Society for Microbiology, Washington, DC.
- McClain, J., D. R. Rollo, B. G. Rushing, and C. E. Bauer. 2002. *Rhodospirillum centenum* utilizes separate motor and switch components to control lateral and polar flagellum rotation. *J. Bacteriol.* **184**:2429–2438.
- Moens, S., K. Michiels, V. Keijers, F. Van Leuven, and J. Vanderleyden. 1995. Cloning, sequencing, and phenotypic analysis of *lafI*, encoding the flagellin of the lateral flagella of *Azospirillum brasilense* Sp7. *J. Bacteriol.* **177**:5419–5426.
- O'Rourke, J., A. Lee, and J. G. Fox. 1992. An ultrastructural study of *Helicobacter mustelae* and evidence of a specific association with gastric mucosa. *J. Med. Microbiol.* **36**:420–427.
- Park, S. Y., B. Lowder, A. M. Bilwes, D. F. Blair, and B. R. Crane. 2006. Structure of FlIM provides insight into assembly of the switch complex in the bacterial flagella motor. *Proc. Natl. Acad. Sci. USA* **103**:11886–11891.
- Poggio, S., C. Abreu-Goodger, S. Fabela, A. Osorio, G. Dreyfus, P. Vinuesa, and L. Camarena. 2007. A complete set of flagellar genes acquired by horizontal transfer coexists with the endogenous flagellar system in *Rhodobacter sphaeroides*. *J. Bacteriol.* **189**:3208–3216.
- Poggio, S., A. Osorio, G. Dreyfus, and L. Camarena. 2005. The flagellar hierarchy of *Rhodobacter sphaeroides* is controlled by the concerted action of two enhancer-binding proteins. *Mol. Microbiol.* **58**:969–983.
- Porter, S. L., G. H. Wadhams, A. C. Martin, E. D. Byles, D. E. Lancaster, and J. P. Armitage. 2006. The CheYs of *Rhodobacter sphaeroides*. *J. Biol. Chem.* **281**:32694–32704.
- Porter, S. L., A. V. Warren, A. C. Martin, and J. P. Armitage. 2002. The third chemotaxis locus of *Rhodobacter sphaeroides* is essential for chemotaxis. *Mol. Microbiol.* **46**:1081–1094.
- Quandt, J., and M. F. Hynes. 1993. Versatile suicide vectors which allow direct selection for gene replacement in Gram-negative bacteria. *Gene* **127**:15–21.
- Shimada, T., R. Sakazaki, and K. Suzuki. 1985. Peritrichous flagella in mesophilic strains of *Aeromonas*. *Jpn. J. Med. Sci. Biol.* **38**:141–145.
- Shinoda, S., and K. Okamoto. 1977. Formation and function of *Vibrio parahaemolyticus* lateral flagella. *J. Bacteriol.* **129**:1266–1271.
- Simon, R., U. Priefer, and A. Pühler. 1983. A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in Gram-negative bacteria. *Biotechnology* **1**:37–45.
- Sistrom, W. R. 1962. The kinetics of the synthesis of photopigments in *Rhodospseudomonas sphaeroides*. *J. Gen. Microbiol.* **28**:607–616.
- Sockett, R. E., J. C. A. Foster, and J. P. Armitage. 1990. Molecular biology of the *Rhodobacter sphaeroides* flagellum. *FEMS Symp.* **53**:473–479.
- Tang, H., and D. F. Blair. 1995. Regulated underexpression of the FlIM protein of *Escherichia coli* and evidence for a location in the flagellar motor distinct from the MotA/MotB torque generators. *J. Bacteriol.* **177**:3485–3495.
- Tans-Kersten, J., H. Huang, and C. Allen. 2001. *Ralstonia solanacearum* needs motility for invasive virulence on tomato. *J. Bacteriol.* **183**:3597–3605.