Secondary bacterial flagellar system improves bacterial spreading by increasing the directional persistence of swimming

Sebastian Budendorfer\textsuperscript{a,b,c,1}, Mihaly Koltai\textsuperscript{a,d,1}, Florian Rossmann\textsuperscript{a,b}, Victor Sourjik\textsuperscript{a,d}, and Kai M. Thormann\textsuperscript{a,b,2}

\textsuperscript{a}Max Planck Institute for Terrestrial Microbiology and LOEWE Research Center for Synthetic Microbiology (SYNMICRO), 35043 Marburg, Germany; \textsuperscript{b}Institute for Microbiology and Molecular Biology, IFZ Interdisciplinary Research Centre, Justus Liebig University Giessen, 35392 Giessen, Germany; \textsuperscript{c}Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, 39065 Hannover, Germany; and \textsuperscript{d}Zentrum für Molekularbiologie der Universität Heidelberg, DKFZ-ZMBH Alliance, 69120 Heidelberg, Germany

As numerous bacterial species, \textit{Shewanella putrefaciens} CN-32 possesses a complete secondary flagellar system. A significant sub-population of CN-32 cells induces expression of the secondary system under planktonic conditions, resulting in formation of one, sometimes two, filaments at lateral positions in addition to the primary polar flagellum. Mutant analysis revealed that the single chemotaxis system primarily or even exclusively addresses the main polar flagellar system. Cells with secondary filaments outperformed their monopolarly flagellated counterparts in spreading on soft-agar plates and through medium-filled channels despite having lower swimming speed. While mutant cells with only polar flagella navigate by a “run-reverse-flick” mechanism resulting in effective cell realignments of about 90°, wild-type cells with secondary filaments exhibited a range of realignment angles with an average value of smaller than 90°. Mathematical modeling and computer simulations demonstrated that the smaller realignment angle of wild-type cells results in the higher directional persistence, increasing swimming efficiency both with and without a chemical gradient. Taken together, we propose that in \textit{S. putrefaciens} CN-32, cell propulsion and directional switches are mainly mediated by the polar flagellar system, while the secondary filament increases the directional persistence of swimming and thus of spreading in the environment.

bacterial motility | cell reorientation | CheY | lateral flagella

The ability to actively explore and exploit the environment provides a major advantage for all kinds of organisms, including bacteria (1, 2). Among bacteria, flagella are common and efficient organelles of locomotion that consist of long, helical, proteinaceous filaments extending from the cell’s surface and are rotated by a membrane-embedded motor to which they are attached by the flexible hook structure. The majority of flagellar motors function in a bidirectional fashion and can rotate either counterclockwise (CCW) or clockwise (CW) (3, 4). Most bacterial species navigate using a random walk that originates from an alternation of straight runs and cell reorientations. In the absence of gradients, such random walk results in a uniform spreading in the environment. In gradients of environmental stimuli, bacterial random walk becomes biased, whereby cells use temporal comparisons of the stimulus strength to suppress reorientations while swimming in a favorable direction. This behavior is controlled by one or more chemotaxis systems, which transduce environmental stimuli to control flagellar motors (5). Signals perceived by an array of sensor proteins are converted into the phosphorylation state of a soluble signal-transmitting protein, CheY. Phosphorylated CheY can directly interact with the flagellar motor and induce a switch in rotation or a motor break. In peripherically flagellated bacteria with several filaments, such as the paradigm system of \textit{Escherichia coli}, CCW rotation leads to formation of a flagellar bundle that drives the cell run. A switch to CW rotation of one or several motors is followed by disassembly of the bundle, leading to reorientation of the cell (“tumble”) and a change in the swimming direction upon resuming CCW rotation of flagella (6, 7). However, numerous bacterial species are polarly flagellated, which results in a pattern of swimming that is different from that of \textit{E. coli}. Recent studies on \textit{Vibrio alginolyticus} that swims using single polar flagellar filament demonstrated that the filament drives the cell forward when rotating CCW but pulls the cells backward when switching to CW rotation. Cell reorientation occurs through rapid cell realignment (“flick”), which is mediated through a buckling instability of the flagellar hook upon resuming CCW rotation. The “run-reverse-flick” realignment occurs in an angle of about 90° and allows efficient spreading and chemotaxis of \textit{Vibrio} and likely also \textit{Pseudomonas} species (8–10).

In addition to a primary polar flagellar system, a number of bacterial species, including \textit{Aeromonas}, \textit{Azospirillum}, \textit{Rhodobacter}, \textit{Shewanella}, or \textit{Vibrio} spp., possess a distinct secondary flagellar system (11, 12). Several previous studies have provided evidence that this secondary system is induced under conditions of increased viscosity or on surfaces, leading to the formation of numerous lateral flagella. For \textit{Vibrio} species, a single polar filament is advantageous for rapid swimming under planktonic conditions, while the lateral set of flagella provides superior performance for swimming or for swimming under viscous conditions (13, 14).

Significance

Flagella-mediated motility is an important or even crucial propagation factor for many bacteria. A number of polarly flagellated species possess a distinct secondary flagellar system, which, as current models suggest, allows more effective swimming under conditions of elevated viscosity or across surfaces. In this study, we demonstrate that such a secondary flagellar system may also exert beneficial effects in bacterial propagating by increasing the directional persistence through lowering the cellular turning angles. The strategy of increasing directional persistence to improve animal spreading efficiency has been proposed previously by theoretical modeling, and here we provide a specific example of how this strategy is used by bacteria.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

\textsuperscript{1}S.B. and M.K. contributed equally to this work.\textsuperscript{2}To whom correspondence should be addressed. Email: Kai.Thormann@mikro.bio.uni-giessen.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1405820111/-/DCSupplemental.
have recently demonstrated that cells of the species *Shewanella putrefaciens* CN-32 possess a functional secondary system that is highly homologous to those identified in *Aeromonas hydrophila* and *Vibrio* sp. (15). Notably, we observed that, in a significant fraction of the cells, the secondary flagellar system is already induced under planktonic conditions in complex media, leading to the formation of a single or sometimes two additional filament(s) at a lateral position at the cell’s surface. Here, we show that these additional filaments function in enhancing efficient spreading and chemotaxis of the cells by increasing the directional persistence.

**Results**

**Swimming Cells with Secondary Filaments Spread Faster than Cells with Polar Flagella only.** Our previous experiments strongly indicated that CN-32 cells possessing a secondary flagellar system cover significantly larger distances in soft agar plates than cells with polar flagella only (15). To further elucidate a potential beneficial role of additional flagellar filaments in efficient spreading, we analyzed expression and production of the secondary flagellar systems within the population. To this end, we used a strain in which the flagellar motor protein FliM2, a component only occurring in flagellar basal bodies of the lateral secondary system, was functionally fused to sfGFP. Cells producing the FliM2-sfGFP fusion protein were placed on soft-agar plates, and after formation of a visible halo caused by radial expansion of the bacterial population, samples were taken from the lateral extension zone with increasing distance to the center (Fig. 1). Expression and localization of FliM2-sfGFP was determined by fluorescence microscopy. Close to the center of the lateral extension zone, about 50% of sampled cells displayed green fluorescent foci at various lateral positions within the cell. This portion of FliM2-sfGFP-producing cells was similar to that observed in planktonic cultures. In contrast, all cells isolated from the fringes of the swimming halos were found to produce FliM2-sfGFP with three fluorescent foci on average at various lateral positions. Flagellar staining revealed that these cells possessed one or sometimes two additional filaments (Fig. S1), and the additional FliM2-sfGFP foci likely represented incomplete secondary flagellar complexes.

In a complementary approach using soft-agar plates, we directly compared the spreading performance of CN-32 cells with or without functional secondary flagella. To this end, we constructed a strain in which we deleted the genes encoding the flagellin subunits of the secondary flagellar system, flaA2 and flaB2. Flagellar staining and subsequent microscopy revealed that ΔflaAB2 cells exclusively formed single polar flagellar filaments. Growth of the percentage of swimming cells of both strains were almost identical. To enable discrimination between wild-type and ΔflaAB2 cells by fluorescence microscopy, both strains were fluorescently tagged by chromosomal integration of constitutively expressed *gfp* or *mCherry*. Then 1:1 mixtures of exponentially growing GFP/mCherry-producing mutant and wild-type cells were allowed to spread in soft agar for 16 h. Samples were taken at different distances from the center of the radial extension zone, and the ratio of the wild-type and mutant cells was quantified by fluorescence microscopy (Fig. 1). We observed that the larger the distance relative to the center of the lateral extension zone which was covered by the cells, the further the ratio of both strains was shifted toward the wild type. At the fringes of the swimming zones, more than 90% of the population consisted of wild-type cells. Filament staining and microscopy revealed that the vast majority of the cells in the sample from the outer rim possessed a single lateral filament in addition to the primary polar one (Fig. S1).

To further determine whether the observed advantage of cells with a secondary flagellar system is not restricted to conditions occurring in soft-agar plates, we conducted a similar spreading competition experiment using chambers that consist of two reservoirs connected by a channel. One reservoir was inoculated with a 1:1 mixture of GFP/mCherry-producing wild-type and ΔflaAB2 cells. After 12 h of incubation, samples were taken from

![Fig. 1.](https://www.pnas.org/doi/10.1073/pnas.1405820111)

Fig. 1. Cells with synchronously functional polar and lateral flagellar systems outperform mutants with polar flagella only in both soft-agar and liquid medium. (A) Representation of CN-32 cells’ radial extension in soft agar (0.25%); 3 μL of exponentially growing cultures of the corresponding strains were allowed to spread for 16 h. Numbers in boxes mark the corresponding sampling areas (1, center; 2, intermediate; 3, rim). (B) Micrographs of CN-32 *flim2*-sfgfp cells isolated from sampling area 1 (Left) and sampling area 3 (Right). Scale bars represent 5 μm. (C) Percentage of fluorescently labeled wild-type and ΔflaAB2-mutant cells in samples isolated from the corresponding sampling areas; 1:1 mixtures of mCherry-labeled wild type and Gfp-labeled mutant (and vice versa) were used to inoculate the plate. (D) Percentage of fluorescently labeled wild-type and ΔflaAB2-mutant cells after traveling from reservoir 1 (R1) to reservoir 2 (R2) through a medium-filled channel. R1 was seeded with 1:1 mixtures of the wild type, and wild type and mutant, respectively, and incubated for 16 h. The error bars represent Sds from at least three samples out of two independent experiments each.
The Presence of Lateral Flagella Affects the Directional Changes. The results of the competition experiments demonstrated that the presence of one or two additional secondary flagellar filaments enhances spreading of CN-32 cells. Preliminary experiments have demonstrated that the presence of the secondary system does not provide an increase in swimming speed (15). To determine whether this is also the case for cells that have covered the greatest distance in soft agar, we determined the swimming speeds of ΔflaAB2 and wild-type cells that were isolated from the fringes of the swimming halos formed on soft-agar plates (Fig. S2). The population of wild-type cells with additional lateral filaments had a velocity of 46.95 ± 14.52 μm·s⁻¹ and were significantly slower than ΔflaAB2 cells which exhibited swimming speeds of 57.30 ± 17.18 μm·s⁻¹. Thus, an increase in velocity could be excluded as the reason for the beneficial role of the secondary flagellar system in swimming motility. However, while recording cell trajectories for measuring swimming speeds, we noticed that cells with lateral flagellar filaments exhibited marked differences in their movement patterns compared with cells with polar filaments only. Cells with single polar flagella periodically (about 10 s) switched from forward to backward swimming. Under the conditions tested, the time interval for backtracking was short (0.3 s), in which time the cells covered less than 5 μm distance. Upon resuming forward movement, quick cellular realignments occurred at a range of angles that centered at 90° (Fig. 2). Thus, swimming of monopolarly flagellated S. putrefaciens CN-32 cells apparently follows the “forward-reverse-flick” pattern that has recently been described for Vibrio species (8, 9). A similar forward-backward movement with respect to time and distance intervals was observed for cells with secondary lateral filaments. However, the directional changes upon resuming forward movement occurred at a much wider array of angles, with an average turning angle below 90° (Fig. 2). In addition, the average period between directional switching events increased to about 20 s. We proposed that these two factors, smaller average turning angle and longer runs, may benefit spreading of wild-type cells by increasing directional persistence of swimming, i.e., correlation in the swimming direction over time.

Main Propulsion and Directional Switches Are Mediated by the Primary Flagellar System of S. putrefaciens CN-32. We further conducted a complementary set of experiments in which we determined the potential interaction of the chemotaxis system with the two different flagellar motors. According to the genome data, S. putrefaciens CN-32 has a single chemotaxis system with a broad sensory repertoire represented by 37 putative methyl-accepting chemotaxis sensor proteins. To dissect the chemotaxis pathway in CN-32, we determined the effect of defined mutants in CheY on the two potential receiving motor systems. To this end, we constructed a constitutively active version of CheY (cheYΔD12K/Y105SW; CheY-GOF “gain of function”) and a non-active version (cheYΔD56N; CheY-LOF; loss of function) (16, 17). The mutated cheY versions were introduced into CN-32 wild type and the mutant backgrounds ΔflaAB1 and ΔflaAB2. In addition, a cheY deletion (ΔcheY) was constructed in all three strains. ΔcheY and CheY-LOF mutations in the wild-type background resulted in straight forward-swimming cells, and almost no directional changes were observed in planktonic cultures. In contrast, in cells bearing a CheY-GOF mutation, the average period between forward and backward movements was drastically shortened (<4 s compared with ~20 s for wild-type cells). All strains were characterized for their ability to navigate in soft-agar plates (Fig. 3).

Wild-type cells bearing ΔcheY, CheY-LOF, or CheY-GOF mutations exhibited a drastically reduced radial expansion. Notably, the level of lateral extension in all three mutants was almost identical to that of a ΔflaAB1 mutant. Furthermore, cells lacking the ability to form the primary polar filament (ΔflaAB1)
were not further affected in swimming motility by additional mutations in or loss of CheY. In contrast, the same CheY mutations introduced into strain background ΔflaAB2, which lacks the secondary system, resulted in cells that were no longer (ΔflaAB2, ΔcheY; ΔflaAB2 CheY-LOF) or just barely (ΔflaAB2, CheY-GOF) capable of navigating through soft agar. Thus, the observed radial expansion of the cheY mutants on soft agar plates was mainly or exclusively conferred by the secondary lateral system.

In addition, we used light microscopy on ΔflaABΔ mutants to identify potential differences in swimming behavior due to loss or mutation of CheY. All actively swimming cells were observed to move in irregular patterns, likely due to the lateral position of the flagellar filament, and never switched from forward to backward movement. Cells that were tethered to the glass surface by the lateral flagellar filament displayed constant CCW rotation, and we did not observe directional switches in any of the strains tested (Movie S1). Based on these results, we concluded that CheY predominantly or even exclusively interacts with the primary polar motor. Thus, main propulsion and chemotaxis-induced forward-backward movements are mediated by the primary polar flagellar system. On the other hand, the lateral system has a role in confining the cellular reorientation to smaller angles.

**Computational Model of Spreading of Shewanella Wild-Type and Mutant Cells.** To determine whether the observed differences in swimming behavior are sufficient to explain the observed advantage in spreading, we performed a mathematical analysis and computer simulations of motility and chemotaxis of wild-type vs. mutant cells. The movement of cells in a uniform environment without gradients can be described analytically as a 2D correlated random walk (18, 19). In this approximation, the mean square displacement (MSD) of the population after time $t$, $R(t)$, can be obtained from the autocorrelation function of the velocity:

$$\langle v(t)v(0) \rangle = \exp(-\lambda + 2D_r t) \frac{v^2}{\lambda t} \exp(\lambda t)$$

$$= \frac{v^2}{\lambda t} \exp(\lambda (1 - \gamma) t) + 2D_r \frac{v^2}{\lambda t} \exp(\lambda (1 - \gamma) t) + 2D_r \frac{v^2}{\lambda t} \exp(\lambda (1 - \gamma) t) \exp(\lambda (1 - \gamma) t)$$

where $\lambda$ is the turning rate, i.e., reciprocal of the mean run duration, $D_r$ is the coefficient of rotational diffusion, $v$ is the speed of swimming, and $\gamma$ is the persistence factor of the movement, i.e., the mean of the cosine of the turning angles, $\gamma = \langle \cos(\Theta) \rangle$. Here we assumed an exponential distribution of run durations and neglected the short backtracking movement of S. putrefaciens CN-32 following runs. Double integration in time on Eq. 1 then gives the value of the MSD:

$$R(t) = \frac{2 v^2}{\lambda} \left\{ \exp[\lambda (1 - \gamma) t + 2D_r t - 1] \right\} + \frac{2 v^2}{\lambda} \exp(\lambda (1 - \gamma) t)$$

Since the exponential term in the numerator goes to zero on the relevant timescale of the experiments (hours), Eq. 2 simplifies to:

$$R(t) = \frac{2 v^2}{\lambda} \exp(\lambda (1 - \gamma) t)$$

Eq. 3 shows that the value of $R(t)$ increases at higher values of the persistence factor $\gamma$, as proposed already in a previous theoretical study describing insect movement (18). The lower average turning angle observed for the wild-type cell movement can thus yield higher persistence and lead to faster spreading. This conclusion was confirmed by calculating the root mean square distance (RMSD) for the mutant and wild-type strains using the full expression of Eq. 2 and the experimentally determined parameter values (Fig. 4A and Fig. S4).

In addition to this analytical calculation, we performed numerical simulations taking into account backward runs. The values of turning angles were generated by assigning discrete probability values to the experimentally measured angles and binning randomly generated numbers by the probability intervals. Durations of individual backward and forward runs were generated using Monte Carlo simulations as in Gillespie’s algorithm (20), assuming exponential probability distributions with the experimentally determined means. Numerical simulations confirmed that the higher persistence factor of the wild-type cells’ movement yields more efficient spreading, if the other parameters have identical values (Fig. 4B and Fig. S4). Although the experimental values of the run duration and swimming speed are different for the wild-type compared with mutant cells, the effects of their longer run periods and lower speed are mutually compensatory, as can be calculated from Eq. 2 (Fig. 4A and Fig. S4) and confirmed by numerical simulations (Fig. 4B and Fig. S4). Therefore, ~90% of the difference in the RMSD results from the higher persistence of the wild-type movement. While the calculated difference in the RMSD is rather small, it yields a consistent increase in the ratio of wild-type to mutant cells at the edge of the simulated spreading population (Fig. 4C), similar to that observed experimentally (Fig. 1C).

Such enhancement of cell spreading in uniform environments might thus alone explain the benefit conferred by the lateral flagella. Nevertheless, higher persistence of movement has also been proposed to have a positive effect on the chemotactic movement of bacteria in gradients (21–23). We thus simulated the effect of the difference in the measured turning angle distribution on the chemotactic movement using the phenomenological model of chemotaxis described in Loci (22). This model does not require knowledge of detailed biochemical parameters and assumes that the pathway response to weak stimuli (in shallow gradients) can be described as a convolution integral of the stimulus history with the impulse response (24, 25). Although the impulse response function was measured for E. coli (see details in SI Materials and Methods), it is believed to be generally required for bacterial chemotaxis (26) and should thus be applicable for S. putrefaciens CN-32. We further varied the time window for sensing and the gradients’ steepness, to investigate the effect of persistence under different conditions.

Wild-type cells indeed showed faster chemotactic movement in shallow attractant gradients (Fig. 4D), suggesting that under these conditions, the observed higher persistence of movement is sufficient to enhance chemotaxis. This difference became negligible in steeper gradients (Fig. 4D and Fig. S4), presumably because already short directional runs in steep gradient enable cells to experience strong chemotactic stimulation. Moreover, the model used here is likely to become imprecise in steep gradients. The positive effect of persistence on the chemotactic efficiency is also diminished by the increase in the run time (Fig. S4), because during longer runs rotational diffusion results in the loss of directional correlation. The exact relation between the run time and the benefit of persistence depends on the value of the coefficient of rotational diffusion, which is not known exactly for S. putrefaciens CN-32.

**Discussion**

For numerous bacterial species, flagella-mediated motility is an important if not crucial factor for successful propagation. Different types of flagellation provide advantages under different environmental conditions, and it has been speculated that some species have maintained two complete flagellar systems to allow more effective motility under a wider range of conditions than could be provided by a single system only (11). In this study, we have provided evidence that a secondary lateral flagellar system...
may not only add propulsion forces for viscous environments or for swarming across surfaces but also enables more efficient spreading under conditions where polar flagella would be sufficient for swimming.

It was shown previously that cells of *S. putrefaciens* are capable of highly efficient chemotactic swimming that, for example, allows cells to successfully track motile marine algae (27). Here, we have demonstrated that *S. putrefaciens* CN-32 and presumably other *Shewanella* sp. most likely navigate by a run-reverse-flick mechanism as has recently been proposed to mediate efficient chemotaxis in *Vibrio* species (8, 9, 28). *S. putrefaciens* CN-32 with a single polar filament exhibit cellular reversals and quick cellular rearrangements by an angle of ~90° upon resuming forward swimming. Under the conditions tested, e.g., with little or no gradient of attractants or repellents, the full run-reverse-flick three-step cycle occurred in less than 0.1 s. In contrast, the secondary lateral flagella function in a unidirectional fashion and were only observed to exhibit CCW rotation, as has similarly been described in an earlier study on the lateral system of *V. alginolyticus* (29). However, while, in this species, CheY is equipped with secondary flagellar systems. Some findings in previous studies indicate that this might be the case: The expression of a secondary flagellar system of *Bradyrhizobium japonicum* planktonic cultures in planktonic cultures has been described in an earlier study on the lateral system of *V. parahaemolyticus* (8, 9, 28).

Using mathematical modeling and computer simulations, we propose that the resulting lowering of the turning angle distribution of a bacteria’s movement leads to more efficient spreading and chemotaxis due to higher directional persistence. Our results are consistent with previous theoretical studies (18) but provide a specific example of how this strategy is used by bacteria. We expect that this function of lateral flagella will be similarly applicable to many of the other numerous bacterial species that are equipped with secondary flagellar systems. Some findings in previous studies indicate that this might be the case: The expression of a secondary flagellar system of *Bradyrhizobium japonicum* planktonic cultures in planktonic cultures has been described in an earlier study on the lateral system of *V. parahaemolyticus* (8, 9, 28).
demonstrated (30), and *V. alginolyticus* strains lacking the lateral flagellar system exhibit a reduced radial extension in soft-agar assays similarly as observed for *S. putrefaciens* CN-32 (31). Given the heterogeneity in steepness of nutrient gradients in many habitats such as marine environments (32), spreading of numerous bacterial species would benefit from an increase in directional persistence conferred by secondary lateral flagella.

**Materials and Methods**

**Bacterial Strains.** The bacterial strains and plasmids that were used in this study are summarized in **Table S1** and **Table S2**. Construction of plasmids and strains was essentially carried out as previously described (15, 33) using oligonucleotides listed in **Table S3**. Detailed information is provided in **SI Materials and Methods**.

**Motility Assays.** Motility of *S. putrefaciens* CN-32 wild-type or mutant single cells or the spreading of cell cultures were monitored using liquid cultures or soft-agar plates, respectively, essentially using protocols that were established earlier (15). Liquid-culture motility assays were performed using early exponential phase cultures of *S. putrefaciens* CN-32. To this end, 1 × 10^7 cells of an overnight culture were added to fresh medium and were grown to an OD_600 of 0.3–0.4 at 30 °C. From this culture, 400 μL were used for immediate microscopic analyses using a Leica TCS SPS (Leica Microsystems) confocal laser scanning microscope equipped with a resonance scanner at 27 frames per second. Single cells were tracked and velocities calculated by measuring track lengths per time. The angle of reorientation events was determined for cells that remained in the focal plane prior and after the directional change occurred.

Soft-agar plates had an agar concentration of 0.25% (wt/vol), and 3 μL of *S. putrefaciens* CN-32 culture were spotted for a motility assay. The plates were incubated for an adequate amount of time at 30 °C, and the radial extension of the cultures was documented. To be able to compare the radial extension of different mutant strains with that of wild-type *S. putrefaciens* CN-32, the appropriate cultures were always spotted onto the same soft-agar plate. Comparative motility performance assays were also performed in μ-Slide VI.0.1 ibiTreat chambers (Ibidi GmbH). Cells from the late exponential growth phase were washed and dissolved in fresh medium containing 15 μg mL^-1^ chloramphenicol to inhibit bacterial growth. 50 μL 1:1 mixtures of appropriately labeled wild-type and Δ flaAB mutant cells were loaded in one of the wells and the chambers were incubated for 16 h at room temperature. Then samples were taken from the second reservoir and characterized accordingly.

**Flagellar Staining.** Staining and microscopy of flagellar filaments was essentially performed as described earlier (see **SI Materials and Methods**).

**Fluorescence Microscopy.** Before fluorescence microscopy, the strains of interest were cultured to midexponential phase or were isolated from soft-agar plates by pipetting. Between 1 and 2 μL of diluted cultures were added on top of an agarose-pad to immobilize cells. An Axio Imager.M1 fluorescence microscope (Zeiss) equipped with a Zeiss Plan Apochromat 100×/1.4 DIC objective was used to visualize single cells. Image acquisition and processing was carried out using the Metamorph 7.5.4.0 software (Molecular Devices). At least 300 cells per data point were evaluated.

**Numerical Simulations.** Numerical simulations of bacterial swimming were performed using custom-written MATLAB scripts as described in **SI Materials and Methods**.

**ACKNOWLEDGMENTS.** This work was supported by Grants TH 831/5-1 and SO 42172-1 from the Deutsche Forschungsgemeinschaft (DFG) within the framework of the DFG Priority Programme SPP1617, the Max Planck Society, and the European Research Council (Advanced Grant 294761-MiRoBE).