Investigating Flagella-Driven Motility in *Escherichia coli* by Applying Three Established Techniques in a Series

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Abstract

Motility is crucial to the survival and success of many bacterial species. Many methodologies exist to exploit motility to understand signaling pathways, to elucidate the function and assembly of flagellar parts, and to examine and understand patterns of movement. Here we demonstrate a combination of three of these methodologies. Motility in soft agar is the oldest, offering a strong selection for isolating gain-of-function suppressor mutations in motility-impaired strains, where motility is restored through a second mutation. The cell-tethering technique, first employed to demonstrate the rotary nature of the flagellar motor, can be used to assess the impact of signaling effectors on the motor speed and its ability to switch rotational direction. The “border-crossing” assay is more recent, where swimming bacteria can be primed to transition into moving collectively as a swarm. In combination, these protocols represent a systematic and powerful approach to identifying components of the motility machinery, and to characterizing their role in different facets of swimming and swarming. They can be easily adapted to study motility in other bacterial species.

Keywords

Biology; Issue 159; bacteria; chemotaxis; *Escherichia coli*; flagella; rotary motor; motility; swimming; swarming; surface sensing; surface motility

Introduction

Bacteria employ many appendages for movement and dispersal in their ecological niches¹. Flagella-driven motility is the fastest of these, promoting the colonization of favorable locales in response to environmental signals, and contributing significantly to the pathogenic ability of some species^{2,3}. Flagellated bacteria can swim individually in bulk liquid, or swarm as a collective over a semi-solid surface⁴. Extracellular flagella attach to and are driven by rotary motors embedded in the membrane, which harness the power of ion gradients to generate torque that causes rotation^{1,2,4,5,6,7,8}. In *E. coli*, whose motors run at

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Disclosures

The authors have nothing to disclose.

Video 1: Rotation of tethered *E. coli* cells. [Please click here to download this video.](#)

Video 2: An active *E. coli* swarm filmed under 60x magnification, demonstrating its characteristic swirling motion behind the edge of the moving front. [Please click here to download this video.](#)

a constant torque⁹, the motor output can be categorized in terms of rotational speed and switching of the rotor between counter-clockwise (CCW) and clockwise (CW) directions. CCW rotation promotes formation of a coherent flagellar bundle that propels the cell forward (run), while a transient switch in rotational direction (CW) causes the bundle to disassemble either partially or fully¹⁰, and the cell to reorient its swimming direction (tumble). *E. coli* typically run for a second and tumble for a tenth of a second. Switching frequency of the rotor or 'tumble bias' is controlled by the chemotaxis signaling system, wherein transmembrane chemoreceptors detect external chemical signals and transmit them via phosphorelay to the flagellar motor to extend runs in response to attractants, or suppress them in response to toxic chemicals^{11,12}. Swimming motility is assayed in 0.3% soft agar.

During swarming, bacteria navigate on a semi-solid surface as a dense collective, where packs of bacteria stream in a continuous swirling motion^{2,13,14,15}. *E. coli* swarms exhibit altered chemosensory physiology (lower tumble bias), higher speeds, and higher tolerance to antimicrobials over cells swimming in bulk liquid^{16,17}. Swimmers vary in their deployment of a plethora of strategies that aid movement, including surfactant production, hyperflagellation, and cell elongation². Swarming offers bacteria a competitive advantage in both ecological and clinical settings^{18,19,20}. There are two categories of swarming bacteria: temperate swimmers, which can swarm only on media solidified with 0.5–0.8% agar, and robust swimmers, which can navigate across higher agar concentrations²¹.

A variety of assays exist to interrogate swimming motility and its regulation. When impaired by mutations or environmental conditions, motility itself offers a strong selection for identifying gain-of-function suppressor mutations. These suppressors can be genuine revertants of the original mutation, or pseudo-revertants, where a second mutation restores functionality. Such mutants can be identified by whole genome sequencing (WGS). An alternative to unbiased suppressor selection is a biased targeted mutagenesis strategy (e.g., PCR mutagenesis). These methodologies often shed light on the function or environmental regulation of the motility apparatus. If the goal is to study motor function, then the restoration of wild-type motility as measured in soft agar may not necessarily indicate restoration of wild-type motor output. The cell-tethering assay, in which cells are attached to a glass surface by a single flagellum and rotation of the cell body is subsequently monitored, can be the initial assay of choice for assessing motor behavior. Although more sophisticated methodologies are now available to monitor motor properties, the required high-speed camera set-up and application of software packages for motion analysis limit their widespread use^{22,23,24,25}. The cell-tethering assay requires only that the flagella be sheared, allowing attachment of the short filaments to a glass slide, followed by videotaping the rotation of the cell body. Although the recorded motor speeds are low in this assay because of the high load the cell body exerts on the flagellum, this assay has nonetheless contributed to valuable insights into chemotactic responses^{26,27,28,29}, and remains a valid investigative tool as discussed below.

Swarming motility poses a different set of challenges to researchers. Selection of gain-of-function suppressors only works in swimmers that produce copious surfactants and swarm readily¹³. Surfactant non-producers such as *E. coli* are fastidious with respect to the choice of agar, media composition and humidity of the environment^{2,13,14,21}. Once

swarming conditions are established, the border-crossing assay¹⁷ is a useful methodology to interrogate the ability of a swarm to navigate new/harsh conditions. Though the protocols presented below relate to *E. coli*, they can be readily adapted for application in other species.

Protocol

1. Isolation of suppressor mutants in motility-deficient strains

NOTE: Use this method as a broad ‘catch-all’ to identify the general nature of the motility defect.

1. Soft-agar plate preparation—NOTE: Soft-agar, also referred to as motility- or swim-agar, is a low percentage agar (~0.2–0.35% w/v), long used to assay chemotaxis^{31,32}.

1. Add 3 g of bacto-agar (0.3% w/v) and 20 g of LB to a 2 L round bottom flask. Add 1 L of ddH₂O (double-distilled water) to the flask and evenly mix the suspension using a stir rod and magnetic stirring plate.
2. Autoclave for 20 min at 121 °C.
3. Allow to cool with gentle agitation using the rod/plate as above. When the temperature reaches approximately 50 °C, pour 25 mL into sterile Petri dishes (100 mm x 15 mm), and allow the molten agar to set with lid in place for at least 1 h, for use within 16 h.

2. Culture preparation, inoculation, and isolation of suppressor mutants—

NOTE: *E. coli* inoculated in the center of nutrient rich media solidified with soft agar consume nutrients locally, creating a nutrient gradient that they follow. As they move outward, defined ‘rings’ appear (Figure 1A), which are related to specific chemoattractants the bacteria respond to. Defects in either the chemotaxis system or structural components of the flagella motor can compromise performance in this assay. Often, mutants with a motility advantage arise during screening, and can be seen emerging from single or from multiple points along the periphery of the ring, from where they ‘flare’ out (Figure 1C). One will notice that the outermost edge of the swimming front contrasts readily against the uncolonized virgin soft agar.

1. Grow overnight cultures of the desired motility-deficient strain in 5 mL of Lennox Broth (LB; 10 g/L tryptone, 5 g/L yeast extract, and 5 g/L NaCl, Table of Materials) at 30 °C with horizontal shaking (220 r.p.m.). The following day, sub-culture (1:100 dilution) in fresh LB, growing under the same conditions to exponential phase (OD₆₀₀ of 0.6).
2. Inoculate 6 µL of the culture into the center of a soft-agar plate (1.1) using a pipette, pushing the loaded sterile tip into the agar to gently expel the contents. Transfer to 30 °C and incubate (Figure 1B), until motility ‘flares’ are evident, emanating from the inoculation point or the periphery of the motility rings, typically in 24–36 h (Figure 1C).

NOTE: In motility assays, inoculate a wild-type strain alongside the mutant isolates for comparison. This wild-type strain will show the characteristic concentric chemotactic rings (Figure 1A) and fill the plate within 8–10 h.

3. Use a sterile wire-loop to lift the cells from the ‘flare’ region and streak to purify single colonies onto an LB hard agar plate (LB prepared as above, solidified with 15 g/L bacto-agar).
4. Pick single colonies from the streak plate using a sterile wire loop and re-purify by streaking for single colonies to ensure isolation of a ‘pure’ colony isolate.

3. Confirmation, and characterization of suppressor mutants

1. Confirm that the isolated suppressor mutant(s) have restored motility. Prepare soft-agar plates (1.1), and cultures for strains of interest (as in 1.2.1), including wild-type and the starting ‘motility-deficient’ strain for comparison.
2. Inoculate the plates (as in 1.2.2) and incubate at 30 °C for 8–10 h.
3. Record the diameter of the outermost ring (edge of the circle) and compare to establish which of the isolates have substantially restored motility.

NOTE: It is recommended that plates be photographed throughout the time-course of the experiment. For best results, use a “bucket of light” device³⁰, where a digital camera is mounted above a light source for better illumination to measure the diameter of the swim colony and distinguish it from uncolonized agar.

4. Subject verified isolates to WGS as required, allowing for sufficient ‘sequence coverage’ to positively identify the mutations that restored wild-type function.

2. Quantifying flagella motor behavior via cell tethering

NOTE: Use this method when normal run-tumble behavior (chemotaxis) appears to be compromised.

1. Culture preparation and flagella shearing

1. Prepare an exponential phase culture of the strain of interest as described in step 1.2.1.
2. Pellet 10 mL of cells by centrifugation at 2,000 $\times g$ for 3 min before resuspending in 10 mL of filter-sterilized Motility Buffer (MB; 10 mM potassium phosphate buffer [0.0935 M K₂HPO₄, 0.0065 M KH₂PO₄, pH 7.0], 0.1 mM EDTA [pH 7.0], 10 mM NaCl, 75 mM KCl).

NOTE: MB supports *motility*, but does not support bacterial growth

3. Repeat step 2.1.2 two more times before resuspending the final pellet in 1 mL of MB.
4. Transfer the cell suspension into a 1 mL syringe and attach a 23G needle to the end. Assemble an identical syringe/needle apparatus and attach the two together

via 6 inches of polyethylene tubing (inner diameter of 0.58 mm) tightly sheathed over each needle tip.

5. Shear the flagella (they are fragile and break easily) by gently passing the cell suspension back and forth from one syringe to the other 50x, with 1 min pauses between every 10 passes.
6. Centrifuge the sheared cells at $2,000 \times g$ for 3 min and resuspend in a final volume of 500 μL of MB.

2. Slide preparation and cell tethering

1. Prepare a cell fixation chamber by stacking an 18 mm x 18 mm coverslip over a 3 inch x 1 inch x 1 mm glass microscope slide, separated by double-sided tape (Figure S1).
2. Flush the chamber with 0.01% (w/v) poly-lysine solution by applying to the top of the chamber. Tilt the bottom edge (the coverslip flush with the microscope slide) onto a task wiper (tissue paper) to help draw solution through the chamber (Figure S1). Then incubate at room temperature for 10 min.
3. Wash the chamber three times with 40 μL of MB using as described in step 2.2.2
4. Add 40 μL of the sheared cell suspension (prepared above) to the top of the chamber and incubate at room temperature for 10 min to allow cells to attach to the coverslip.
5. Gently flush the chamber with 40 μL of MB as in 2.2.3 to remove unattached cells.

3. Cell rotation recording and quantification

1. Transfer the microscope slide loaded with tethered cells to the microscope stage.
2. Using phase-contrast microscopy and a 100x objective, scan the population for cells that are fixed in place, and rotating on a single axis, i.e., smooth rotations on a fixed point rather than presenting at an angle wherein the cell moves in and out of focus (**Video 1**).
3. Use a commercial microscope and associated camera. Open the associated software, ensure cells of interest are in focus and click **video acquisition** to record the cell rotation for one minute (at 10 frames per second or higher).
4. From video playback, quantify the number of complete rotations per minute and the number of times the cell changes direction (switching frequency).

NOTE: Rotational speeds and switching frequency may be too fast to gauge by eye so it is recommended to use video software that offers slow/fine playback, or adopts an automated software system to quantify rotational patterns³³. An alternative would be to increase the viscosity of the MB using methyl cellulose (or similar agent) to help slow and resolve the rotation of faster bacteria or compensate when cameras with low framerates are in use.

5. Repeat step 2.3.2 with biological replicates to compile a representation of the population of interest.

3. Preparation of swarms in a border-crossing assay

NOTE: Use this method for assessing the impact of a mutation or condition on group motility. Swarm-agar refers to agar where the percentage is typically higher than that of soft-agar. In soft agar (0.3 %), cells swim individually inside the agar. In swarm agar (0.5% and above), cells move as a group on the surface. While swarm plates must be used as detailed here, swim plates have a longer shelf life, and may be used for several days. Our personal preference is to use in 1–2 days.

1. Preparation of swarm agar

1. Add 5 g of Eiken agar (0.5% w/v) and 20 g of LB to a 2 L round bottom flask. Add 1 L of ddH₂O to the flask and evenly mix the suspension using a stir rod and magnetic stirring plate.
2. Autoclave for 20 min at 121 °C.
3. Allow to cool with gentle agitation to avoid any air bubbles using the rod/plate as above. When approximately 50 °C, add filter-sterilized glucose for a final concentration of 0.5 %.
4. Pour 25 mL into sterile Petri dishes (100 mm x 15 mm) and allow to set at room temperature for at least 14 h and no more than 20 h. Do not store for future use.

2. Inoculation and incubation of swarm plates

1. Inoculate 6 µL of a mid-exponential culture (prepared as in 1.2) by spotting on top of the agar.
2. Leave the lid off for 5–10 min and replace when the inoculum has dried into the agar surface.
3. Incubate at 30 °C for 8 h. Avoid the temptation to inspect swarm progress by removing the lid, as this will contribute to drying of the agar and impair swarming.

NOTE: Incubation time may vary depending on the strain phenotype. Some isolated mutations may hamper swarming ability and will require a reduced percentage of agar or a more prolonged period of incubation.

3. Preparation of border-crossing assay plates—NOTE: This assay utilizes a modified Petri dish, where a plastic divide (border) creates two chambers, rather than one (Figure 2A). Each chamber can be prepared independently of the other, offering differing conditions for swarming, prior to ‘connecting’ the two. Depending on experimental design, the first chamber (designated left) can be prepared with either swim agar (0.3% w/v) or swarm agar (0.5% w/v) from where the bacteria can migrate across the border into the right chamber containing swarm agar +/- any required supplement or challenge (e.g., antibiotics).

Migration on either agar is typically measured/compared by recording the widest diameter of bacterial colonization (edge to edge) from the original point of inoculation.

1. Prepare swim agar as described in 1.1 if required.
2. Prepare swarm agar as described in 3.1.
3. Pour ~30 mL of swarm agar (with desired supplementation if required) into the right chamber of a dual-compartment Petri dish (100 mm x 15 mm), to the point where it is level with the plastic divider between chambers, but not overflowing into the left (Figure 2B).
4. After the agar has hardened, fill the left chamber with ~30 mL of swim or swarm agar, again to the point of contact with the plastic divide (Figure 2C). Before it sets, use a sterile pipette tip to gently drag the agar over the border to connect the two sides with a ~1 mm tall agar bridge that spans the entire length of the divide (Figure 2D).
5. Allow the plate to dry at room temperature (3.1).

NOTE: An alternative method of creating bridge is allow the left chamber agar to dry and then slowly pipette ~100 μ L of molten swarm agar along the plastic divider to bridge the two chambers (3.4). Inoculate the plates on the left chamber as detailed above for swim (1.2.2) or swarm (3.2) agar, before incubating at 30 °C for 12–16 h, or until swarms have made sufficient progress over the right chamber to allow comparisons between strains of interest.

Representative Results

The isolation of pseudo-revertants in an *E. coli* strain whose motility is impaired by high levels of the signaling molecule c-di-GMP, was detailed in recent work from our lab³⁴. This strain (JP1442) harbored two mutations: *yhjH* and *ycgR*. YhjH is the most active phosphodiesterase that degrades c-di-GMP in *E. coli*. Absence of YhjH leads to elevated c-di-GMP levels and inhibition of motility. YcgR is a c-di-GMP effector. In complex with c-di-GMP, YcgR binds to the flagellar rotor to first induce CCW motor rotation and subsequently decrease motor speed. Cell tethering and bead assays showed that motor behavior returned to normal in the double mutant, yet motility in soft agar did not³⁴. So, we deployed step 1 of the protocol to isolate pseudo-revertant flares in the double mutant (Figure 1C). The majority of the mutations mapped by WGS (HiSeq 4000 platform, PE 2 × 150 setup³⁴) to *rssB*, which codes for a response regulator/adaptor protein that normally directs ClpXP protease to target σ^S for degradation³⁴. One of these revertants, which displayed motility close to wild-type (AW405, compare Figure 1A,D), was used to generate representative results for step 2 and 3 of the protocol section, using as controls both its double mutant parent (Figure 1B) and isogenic wild-type strain (Figure 1A).

For step 2 of the protocol section, video captures were analyzed to calculate rotations per minute (each 360° complete rotation), and CW_{Bias} (the fraction of time motors rotate in a CW direction, or tumble bias). The *yhjH* showed fewer rotations per minute and a lower CW bias compared to the wild-type, as expected (Figure 3). Both the *yhjH ycgR* double mutant and its suppressor showed motor behavior similar to wild-type, observations

supported by a previous analysis using the higher-resolution ‘bead’ assay detailed in the introduction above in previous work³⁴.

For step 3 of the protocol section, the border-crossing assay (Figure 2) was used to compare the abilities of the wild-type and the suppressor isolate, first to swarm, and then to move across the border and swarm on agar supplemented with kanamycin. Results show that both strains reached the border at a similar time (data not shown) indicating similar rates of swarming from an identical inoculation point. However, cross-over of the swarm to the right (antibiotic) chamber was marginally, but consistently greater for the wild-type than the suppressor at 20 µg/mL kanamycin (Figure 4). The difference between the two strains was more pronounced at 40 µg/mL kanamycin. Together, these data suggest that the mutations in *rssB* that restored motility on soft-agar plates (Figure 1D), negatively impact the antibiotic resistance of the suppressor strain during swarming (Figure 4).

Discussion

The isolation and characterization of suppressor mutations have successfully contributed to identifying key components of the chemotaxis system^{35,36,37}, as well as the motor machinery itself^{38,39,40}. While using Protocol 1, it is important to include multiple independent replicates to ensure the isolation of a large spectrum of possible mutations that could compensate for the loss of motility. Increasing the number of bacteria by streaking the culture in a line rather than a spot, can improve the odds of generating suppressors⁴¹. Isolation of the same mutation (as determined by DNA sequencing) multiple times increases confidence in its authenticity. WGS will invariably reveal the presence of other mutations in the genome. It is therefore important to verify the results by transducing (where possible) the identified mutation back into the original motility-deficient background. The suppressor mutant approach is rooted in restoring the function through a secondary mutation, so a limitation of this method is that if a critical structural gene is deleted, i.e., one that underpins the entire pathway or structure, there may be no scope for compensation. Despite being an old method, our recent work³⁴ demonstrates its continuing utility in elucidation of new pathways that contribute to bacterial motility.

For the quantification of motor output, the cell-tethering approach remains a universally accessible tool requiring only a microscope with a camera attachment. Cell tethering has already been used in a diverse number of bacterial species including *Salmonella*⁴², *Pseudomonas*⁴³, *Streptococcus*⁴⁴, and *Rhodobacter*³³. The success of the protocol is largely contingent on proper shearing and attachment of cells. Shearing too aggressively or omitting the pause between shears (2.1.5) tends to promote inconsistent or incomplete shearing of filaments, resulting in non-motile cells or cells tethered on a skewed axis. The enduring relevance of this protocol remains, despite the adoption of the higher-resolution bead assay by many research groups (including ours). The primary limitation of the bead assay comes from the need for the bead to adhere to the filament of the bacteria of interest. This technique has greatly benefitted from studies in *E. coli* that identified a ‘sticky’ flagellin allele, which facilitates adherence⁴⁵. The sticky variant is also superior in the cell-tethering assay. Such a variant is not yet available for the majority of flagellated bacteria. The situation is complicated further with some organisms possessing multiple

flagellin proteins⁴⁶, and in the case of *Vibrio* sp., also possessing a membranous sheath⁴⁷. Cell-tethering can also be performed using a species-specific anti-flagellin antibody or an antibody to an engineered epitope tag.

While bacteria can swim immediately upon introduction into a liquid medium, this is not true of swarming, where cells must be first primed into a swarming state. Surface contact triggers a physiological change required for cells to initiate swarming^{48,49,50,51}, resulting in a lag phase and a buildup of high cell density. Physiological changes include remodeling of the chemotaxis system in *E. coli*¹⁶, and adaptations such as cell elongation and/or hyperflagellation for other bacteria^{13,14,21}. Given the physiological changes required to initiate swarming, we strike a cautionary note about studies that have attempted to mimic select facets of swarming - such as high density or increased cell length - simply by concentrating planktonic cell cultures to increase density, and/or inducing cell elongation by use of antibiotics that inhibit cell division^{52,53}. If using cell elongation as a marker, we caution also that compared to planktonic cells, there is only a marginal increase in the average length of swarmer cells in *Salmonella* or *E. coli*⁵⁴. Swarm assays are harder to establish than swim assays. Variables include the commercial source of the agar used to solidify the media (the special Eiken agar is essential for swarming in *E. coli*, whereas the more standard Difco agar supports swarming for all other bacteria), use of rich versus minimal media (*E. coli* and *Salmonella* require glucose supplementation), and most critically ambient humidity^{2,13,14,21}. Of these, maintaining optimal humidity can be the most frustrating. [An excellent, methodical, optimization for swarming in an organism of choice (*Pseudomonas aeruginosa*) has been demonstrated by Morales-Soto and co-workers⁵⁵.] Swarm media must be sufficiently moist to promote swarming but not so moist as to allow passive spreading/sliding, which can be readily mistaken for active swarming⁵⁶. It is therefore critical that swarms be checked under a microscope to confirm the distinct patterns of movement associated with this collective motility (**Video 2**). Temperature is also an important consideration for optimizing the swarming assay. Higher temperatures, for example 37 °C, will dry out the plates sooner than at 30 °C. Using an incubator with humidity control (~70–80%) can help mitigate these issues, including seasonal changes that could affect internal building temperatures and humidity. Once successfully established, protocol 3 provides a powerful way to investigate one of the most interesting aspects of swarming bacteria, elevated resistance to antibiotics¹⁷. All protocols described here can be applied to new organisms to identify pathways that specify and control flagella mediated motility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1: Soft-agar motility assays and emergence of suppressor flares.

The plates contain LB solidified with 0.3 % w/v agar. *E. coli* strains were inoculated in the center of each plate and incubated at 30°C for 8 h, except for C, which was incubated for 16 h. (A) Wild-type *E. coli* (AW405). (B) Motility-deficient variant *yhjH ycgR* (JP1442). (C) As in B, except longer incubation times. Arrows point to faster moving 'flares' emerging at the peripheral ring of the expanding swim colony. (D) A suppressor isolated from a flare in C. [Please click here to view a larger version of this figure.](#)

Figure 2: Schematic for setting up a Border-crossing plate assay.

(A) Pour ~30 mL of swarm agar (with desired antibiotic) into the right chamber of a divided petri dish until level with the plastic divider and allow to set with lid closed. (B) Fill the left chamber with ~30 mL of swim or swarm agar to the point of contact with the top of the plastic divider. (C) Use a sterile pipette tip to gently drag the molten swarm agar over the border, thereby connecting the two sides with a ~1 mm tall agar bridge and allow to set with lid closed. (D) Allow the plate to dry further at room temperature overnight before inoculating the left chamber with the desired strain, and incubating at 30 °C. [Please click here to view a larger version of this figure.](#)

Figure 3: Motor properties of various strains as measured by the cell-tethering technique. Wild-type (AW405), *yhjH* (VN133), *ycgR yhjH* (JP1442), and its suppressor (JP1836) were grown in LB at 30 °C to mid-exponential phase prior to tethering. (A) Rotations per minute (completed 360° turns), and (B) CW_{bias} (fraction of time motors rotate in a CW direction). Standard deviation of the mean (±). 20 tethered cells were observed for 60 sec in each strain. [Please click here to view a larger version of this figure.](#)

Figure 4: Border-crossing assays.

Mid-exponential phase cultures of wild-type *E. coli* (AW405) and the suppressor mutant (JP1836) were inoculated at the indicated position (*) in the left compartment of the divided plate containing swarm media, and incubated at 30 °C. They reached the border at comparable times. The plates were incubated for a further 6 h, during which the swarm crossed over to the right chamber, in which the media was supplemented with kanamycin (Kan; numbers indicate µg/mL). Plates are representative of three biological replicates each carried out in triplicate. [Please click here to view a larger version of this figure.](#)

Table_of_Materials

Name	Company	Catalog Number	Comments
Reagents			
Bacto Dehydrated Agar	Fisher Scientific	DF0140–15–4	
EDTA Disodium Salt, Dihydrate	Fisher Scientific	02–002–786	
Eiken agar	Eiken Chemical Co. Japan	E-MJ00	Essential for <i>E. coli</i> swarming
Glucose D (+)	Fisher Scientific	410955000	
LB (Lennox) Broth	Fisher Scientific	BP1427–500	
Poly-L-lysine Solution (0.1%)	Sigma-Aldrich	P8920	
Potassium chloride (KCl)	Fisher Scientific	18–605–496	
Potassium Phosphate monobasic (KH ₂ PO ₄)	Fisher Scientific	BP362–500	
Potassium Phosphate dibasic (K ₂ HPO ₄)	Fisher Scientific	BP363–500	
Sodium chloride (NaCl)	Fisher Scientific	S271–500	
Materials and Equipment			
CellSense microscope imaging software (V. 1.6)	Olympus		Or equivalent software for microscope used
Electron Microscopy Sciences Scotch 666 Double Sided Tape	Fisher	50–285–28	
Frosted microscope slides 3×1×1mm	Fisher	12–550–343	
Olympus BX53 microscope	Olympus	BX53	Any upright or inverted phase microscope can be used
Petri dishes (100 mm diameter)	Fisher Scientific	FB0875712	For soft-agar assays
Polyethylene Nebulizer Capillary Tubing (0.58mm x 99mm 3.0m)	Perkin Elmer	9908265	
Round Petri Dish with 2 Compartments	VWR	89200–944	For border-crossing assays
Safety Hypodermic Needles (23G)	Fisher Scientific	14–826A	
Sterile Syringe - 1 mL	Fisher scientific	14–955–450	
Task/Tissue wipes	Fisher scientific	06–666	Or equivalent single use tissue wipes
VWR micro cover-glass 18x18mm	VWR	48366205	
XM10 camera	Olympus	XM10	Or equivalent microscope camera