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

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **No (two different rooms several ft apart)**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Rasika Harshey:** The protocols demonstrated in this video can be reproduced in any lab in the world. In combination, they represent a systematic and powerful approach to characterizing bacterial flagellar motility.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Rasika Harshey:** Motility is a crucial aspect of bacterial dissemination and survival. These three methods are powerful tools for the analysis of individual and collective motility.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Jonathan Partridge:** The techniques discussed here can readily be applied to study an array of bacteria, including those classified as pathogenic in nature.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.4. **Jonathan Partridge:** When attempting this procedure for the first time, keep in mind that if the swarm plates are prepared incorrectly, inconsistent behavior or patterns that mimic, but do not truly reflect, collective motion may be observed.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

2. Isolation of Suppressor Mutants in Motility-deficient Strains

2.1. Grow overnight cultures of the desired motility-deficient strain in 5 milliliters of Lennox Broth at 30 degrees Celsius with horizontal shaking **[1]**. Then, sub-culture in fresh LB under the same conditions to exponential phase **[2]**.

2.1.1. Talent opening the incubator and taking out an overnight culture flask.

2.1.2. Talent transferring some media from the overnight culture to fresh LB.

2.2. Inoculate 6 microliters of the culture into the center of a soft-agar plate, pushing the loaded pipette tip into the agar to gently expel the contents **[1]**. Incubate the plate at 30 degrees Celsius until motility 'flares' are evident **[2-TXT]**, emanating from the inoculation point or the periphery of the motility rings **[3]**.

2.2.1. Talent inoculating the plate.

2.2.2. Talent putting the plate in the incubator. **TEXT 24 – 36 hours**

2.2.3. Plate with motility flares.

2.3. Use a sterile wire-loop to lift the cells from the 'flare' region **[1]** and streak them onto an LB hard agar plate in order to purify single colonies **[2]**.

2.3.1. Talent lifting the cells from the flare region.

2.3.2. Talent streaking the cells onto a hard agar plate.

2.4. To confirm that the isolated suppressor mutants have restored motility, culture them on soft agar plates for strains of interest. Include wild-type and the starting 'motility-deficient' strain for comparison **[1]**.

2.4.1. Agar plates in the incubator.

2.5. After incubating the culture plates at 30 degrees Celsius for 8 to 10 hours **[1]**, record the diameter of the outermost ring to establish which of the isolates have substantially restored motility **[2]**.

2.5.1. Talent opening the incubator and taking the plates out.

2.5.2. Talent measuring the diameter.

3. Quantifying Flagella Motor Behavior via Cell Tethering

3.1. Prepare an exponential phase culture of the strain of interest, then pellet 10 milliliters of cells by centrifugation at 2,000 *x g* for 3 minutes **[1]** and resuspend them in 10

milliliters of filter-sterilized Motility Buffer, or MB **[2]**. Wash the cells two more times in MB **[3]** and resuspend them in 1 milliliter of MB after the final centrifugation **[4]**.

3.1.1. Talent putting the cells in the centrifuge and closing the lid.

3.1.2. Talent resuspending the cells in 10mL of MB.

3.1.3. Talent opening the centrifuge and taking out the cells.

3.1.4. Talent resuspending the cells in 1 mL of MB.

3.2. Transfer the cell suspension into a 1 milliliter syringe **[1]** and attach a 23-gauge needle to the end **[2]**. Assemble an identical syringe and needle apparatus and attach the two together via 6 inches of polyethylene tubing tightly sheathed over each needle tip **[3]**. *Videographer: This step is important!*

3.2.1. Talent transferring the cell suspension into the syringe.

3.2.2. Talent attaching the needle to the syringe.

3.2.3. Talent connecting the two syringes with the tubing.

3.3. Shear the flagella by gently passing the cell suspension back and forth from one syringe to the other 50 times, with 1-minute pauses between every 10 passes **[1]**. Then, centrifuge the sheared cells at 2,000 *x g* for 3 minutes **[2]** and resuspend them in 500 microliters of MB **[3]**. *Videographer: This step is important!*

3.3.1. Talent passing the cell suspension from one syringe to the other.

3.3.2. Talent putting the cells in the centrifuge and closing the lid.

3.3.3. Talent resuspending the cell pellet in MB, with the MB container in the shot.

3.4. To prepare a cell fixation chamber, stack an 18 by 18-millimeter coverslip over a glass microscope slide, separated by double-sided tape **[1]**. Add 0.01% poly-lysine solution to the top of the chamber to flush it **[2]**. *Videographer: This step is important!*

3.4.1. Talent assembling the fixation chamber.

3.4.2. Talent adding the poly-lysine to the top of the chamber.

3.5. Tilt the bottom edge onto a task wipe to draw the solution through the chamber **[1]**, then leave it at room temperature for 10 minutes **[2]**. Wash the chamber three times with 40 microliters of MB **[3]** and add 40 microliters of the sheared cell suspension to the top of the chamber **[4]**.

3.5.1. Talent tilting the bottom edge of the chamber onto a task wipe.

3.5.2. Talent setting the chamber down to incubate.

3.5.3. Talent washing the chamber with MB. *Videographer: Obtain multiple usable takes of this because it is reused in 3.6.1.*

3.5.4. Talent adding cell suspension to the chamber.

3.6. Allow the cells to attach to the coverslip for 10 minutes, then gently flush the chamber with 40 microliters of MB to remove unattached cells **[1]**.

3.6.1. *Use 3.5.3.*

3.7. Transfer the slide to the microscope stage **[1]** and use phase-contrast microscopy and a 100 X objective to scan the population for cells that are fixed in place and rotating on a single axis **[2]**.

3.7.1. Talent positioning the slide on the microscope stage.

3.7.2. SCOPE: Field of view with cells that are fixed in place or rotating on a single axis. Authors: Please upload this to your project page when you have it. **NOTE: Use Video_1.m4v**

3.8. Open the microscope software, make sure that the cells are in focus, and begin video acquisition to record the cell rotation for 1 minute **[1-TXT]**. From video playback, quantify the number of complete rotations per minute and the number of times the cell changes direction **[2]**.

3.8.1. Talent at the computer, opening the software and beginning acquisition. **TEXT: 10 frames/second or higher**

3.8.2. LAB MEDIA: Video of cells. Authors: Please upload this to your project page when you have it. **NOTE: Use Video_2.m4v**

4. Preparation of Swarms in a Border-crossing Assay

4.1. Inoculate 6 microliters of a mid-exponential culture by spotting it on top of the agar **[1]**. Leave the lid off for 5 to 10 minutes and replace it when the inoculum has dried into the agar surface **[2]**, then incubate the plate at 30 degrees Celsius for 8 hours **[3]**.

4.1.1. Talent inoculating the culture onto the plate.

4.1.2. Plate with the lid off, then talent replacing the lid.

4.1.3. Talent putting the plate in the incubator and closing the door.

4.2. Pour approximately 30 milliliters of swarm agar into the right chamber of a dual-compartment Petri dish **[1]**, ensuring that it is level with the plastic divider between chambers but not overflowing into the left **[2]**. *Videographer: This step is difficult and important!*

4.2.1. Talent pouring the swarm agar.

4.2.2. Close up of the swarm agar being poured to the correct level.

4.3. After the agar has hardened, fill the left chamber with approximately 30 milliliters of swim or swarm agar **[1]**. Before it sets, use a sterile pipette tip to gently drag the agar over the border and connect the two sides with a 1-millimeter tall agar bridge that

spans the entire length of the divide **[2]**. Allow the plate to dry at room temperature **[3]**. *Videographer: This step is difficult and important!*

4.3.1. Talent pouring the agar into the other chamber.

4.3.2. Talent creating the bridge between the two chambers with the pipette tip.

4.3.3. Plate drying at room temperature.

4.4. **Jonathan Partridge:** As you pour the agar, pay attention to its depth. If it is too high it will overflow, and if it is too low you will be unable to bridge the two chambers.

4.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Results

5. Results: Motility of Δ*yhjH* Δ*ycgR E. coli* Strain

5.1. This protocol was used to isolate pseudo-revertant flares in a double mutant *E. coli* strain with impaired motility **[1]**. One of these revertants displayed motility close to wild-type **[2-TXT]**. The double mutant parent **[3]** and isogenic wild-type strain were used as controls **[4]**.

5.1.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize the flared in C that the arrows are pointing to.*

5.1.2. LAB MEDIA: Figure 1. *Video Editor: Emphasize A and D. Label A "***Wild Type***" and D "***Revertant***".*

5.1.3. LAB MEDIA: Figure 1. *Video Editor: Emphasize B.*

5.1.4. LAB MEDIA: Figure 1. *Video Editor: Emphasize A.*

5.2. Video captures were analyzed to calculate rotations per minute **[1]** and the fraction of time that motors rotate in a clockwise direction, or tumble bias **[2]**. The *yhjH (pronounce 'Y-H-J-H')* mutant showed fewer rotations per minute and a lower tumble bias compared to wild-type *E. coli*, as expected **[3]**.

5.2.1. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

5.2.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize B.*

5.2.3. LAB MEDIA: Figure 3. *Video Editor: Emphasize yhjH data in both A and B.*

5.3. Both the double mutant and its suppressor showed motor behavior similar to wild-type **[1]**.

5.3.1. LAB MEDIA: Figure 3. *Video Editor: Emphasize yhjH ycgR and suppressor data in both A and B.*

5.4. The border-crossing assay was used to compare the abilities of the wild-type and the suppressor isolate to swarm and then to move across the border and swarm on agar supplemented with kanamycin **[1]**.

5.4.1. LAB MEDIA: Figure 4.

5.5. Both strains showed similar rates of swarming from an identical inoculation point, but cross-over of the swarm to the antibiotic chamber was consistently greater for the wild-type than the suppressor **[1]**. The difference between the two strains was more pronounced at higher kanamycin concentrations **[2]**.

5.5.1. LAB MEDIA: Figure 4.

5.5.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the Kan 40 images.*

Conclusion

6. Conclusion Interview Statements

6.1. **Jonathan Partridge:** The swarm assays can be tricky to set up. You may need to spend some time optimizing the drying conditions to suit your bacteria of interest, as well as your lab conditions during media preparation.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.2.1, 4.2.2.*

6.2. **Jonathan Partridge:** The techniques in this protocol have been applied to an array of flagellated bacteria and can likely be applied to a plethora of untested species.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.