An Inexpensive Way to Record and Quantify Bacterial Swarming

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Abstract

Bacterial swarming refers to a rapid spread, with coordinated motion, of flagellated bacteria on a semi-solid surface[1]. There has been extensive study on this particular mode of motility among microbiologists and biophysicists because of its interesting biological and physical properties (e.g. enhanced antibiotic resistance[2], turbulent collective motion[3]). The existing equipment for recording swarm expansion rate can easily go beyond tens of thousands dollars[4], yet the conditions are not accurately controlled, resulting in large variations across the assays. Here, we report a reliable protocol to perform reproducible bacterial swarming assays and an inexpensive way to record and quantify the swarming activity by time-lapse photography. This novel protocol consists of three main parts: 1) building a “homemade”, environment-controlled photographing incubator; 2) performing bacterial swarming assay; 3) taking serial photos over time and calculating the swarming rate. The homemade incubator is economical, easy to operate, and has wide applications. In fact, this system can be applied for any slow evolving biological process that needs to be monitored by camera under a controlled environment.

Introduction

Coordinated multicellular migration across a moist surface known as bacterial swarming is an important phenotype that has been studied for decades in laboratories, including those inclinical settings. The colony expansion speed and morphological patterns are key parameters to describe bacterial swarming activity. We built an environment-controlled incubator to perform swarming assay. Inside the incubator, a digital camera was mounted on the top to take time-lapse images of the swarming activity. After the recording, images were transferred to a laptop for quantification of the swarming rate. The swarming area can be calculated manually using ImageJ plugins or identified automatically using a python script we developed. The system was tested by over 1,000 swarming events to assure the stability and reproducibility.

At first glance, one may think swarming assay is simple: just inoculate bacteria on an agar petri dish, then taking a picture of the plate after a period of incubation. However, it can be challenging to

perform swarming assays with reproducible results and record high quality images for further analysis. Here we list a few technical challenges one may encounter and provide corresponding solutions:

1. A typical swarming event may take about 10 to 20 hours for the bacteria to cover a 9-cm petri dish. Besides the full coverage time, sometimes we need to know how long the lag phase lasts, by what time branches form at the colony edge, and at the microscopic level, when cell elongation happens. Thus, the researcher needs to check at the plate regularly. Suppose one starts the assay during daytime, he/she may need to scan the plates every 30 minutes over night. Otherwise, he/she may miss the details. In our protocol, time-lapse photography helps to capture the key frames so that the researcher does not have to stay up late or regularly check in order not to miss key events.

2. Bacterial swarming is highly sensitive to the environment. Fluctuations in temperature and humidity will cause large differences in swarming rate and colony pattern. In this case, a stable humidity and temperature-controlled environment is critical for the assay. In our system, we utilized a thermo-insulated tent, a humidity control unit, and a temperature control unit to minimize the environmental fluctuation during the assay. One can readily set different humidity and temperature for swarming strain screening.

3. Since agar gel contains nearly 99% water, condensation readily forms on the plate lid, which obscures photo taking. We designed the incubator in such a way that when the plates are invertedly placed on the platform, the temperature of the lid is slightly higher than that of the agar, which prevents condensation.

4. Taking a clean and clear photo of swarming plates is tricky. The swarming plate has three optical surfaces: the plate lid, the agar surface and the plate bottom. When the camera flashlight or auxiliary front light is used, some light is reflected by the lid and bottom of the plate to the camera, forming unexpected light spots in the photos. In our design, for one plate photo shooting, we used circularly fluorescent backlight plus an adjustable light shield. For multi-petri dish (up to 9) event, LED light strip was used for sidelight illumination. Image quality will be better using the light shield because the light field is well calibrated, but the efficiency is higher when using LED light since it shines on a

larger area to fit 9 petri dishes at a time.

5. Quantification of the swarming rate takes much effort. Usually the researchers scan the plates and measure the radius of the swarm colony using a ruler. For an irregular shape of colony, they usually make a rough estimation of “effective radius”. When multiple plates are used in the assay and one wants to plot the swarming area vs. time instead of just calculating the average swarming rate, it is a lot of work. In our case, developed a python script helped calculate the swarming colony area of each frame automatically. The accuracy was confirmed by ImageJ free selection tool.

Compared with other protocols in this field, our protocol has its distinctive advantages.

(1) **Affordability.** In 2015, Shrout team from University of Notre Dame developed a protocol on preparation, imaging and quantification of swarming assay[4]. However, in their protocol, they used a commercial equipment “Bruker in vivo imaging station”. This product is no longer available from the company and a used one costs over $58,000 (dotmed.com). A lot of microbiology labs may not need many complex functions of the equipment, such as x-ray imaging or in vivo fluorescence imaging. These labs may not afford or be willing to invest such a big amount of money on a bacterial swarming chamber. In contrast, the cost of our system is around $1,000 with everything included.

(2) **Accuracy.** For Bruker imaging station, to maintain humidity, they proposed to place a plate of water on each of the swarming plates. In this way, it is hard to control the humidity within specific range because different amount of water will result in different humidity. Nevertheless, in our design, the chamber humidity is well controlled. One can set the parameters before the assay starts and the environment is controlled dynamically using the digital sensor and controllers.

(3) **Efficiency.** In 2018, independent researcher Maria Cobo developed a time lapse imaging chamber for bacterial colony morphology observation[5] which allows for photography one petri dish a time while the temperature is not controlled. In our case, up to nine 9-cm petri dishes can fit inside the incubator, increasing efficiency. It is not merely having a larger box since adjusting the size of the box will alter the optical path in a subtle way. The geometry of our incubator and light field for photography were well coupled to ensure best lighting. In Dr. Cobo’s protocol, the time-lapse video

flickers a lot, and fog forms on the lid. In our protocol, these problems have been completely fixed. This protocol includes three parts: (1) assembly of the incubator; (2) swarming assay preparation; and (3) image taking and data processing. By strictly following the procedure, we guarantee that one can get a homemade bacteria incubator with stable swarming results and high-quality images as we had in our lab. The overall cost is around $1,000 dollars depending on what camera you use in the system. Indeed, any digital camera that has a “Manual Mode” is adequate for the purpose. Swarming plate preparation is not difficult, but one needs to be very careful on certain details. For instance, bacterial swarming system is very sensitive to small environment perturbation as well as surface conditions such as the roughness of the agar surface. In the photo shooting part, we will show in detail how to tune the camera settings. Finally, we’ll explain how to manually process the data using ImageJ, and also automatically quantify the swarming area using our Python script.

Reagents

Materials

(i) For building the photography incubator

Hydroponic Indoor Garden Grow Tent (24" x 24" x 48", Yaheetech, model no. YT-2801)

Photography unit

Digital camera (Panasonic, model no. DMC-FZ50)

LCD Timer Remote Control (JJC, model no. TMD)

AAA battery (2 pcs, Duracell)

Zinc-plated slotted angle (4 pcs, 1.5’’ x 14 Gauge x 36’’, Crown Bolt)

Zinc-plated slotted angle (10 pcs, 1.5’’ x 14 Gauge x 18’’, Crown Bolt)

Aluminum flat bar (0.75’’ x 36’’ x 0.125’’, Everbilt)

Black polyester cloth (20’’ x 20’’, Dazian)

Bolts and Nuts (40 pairs, M5, Crown Bolt)

Black acrylic sheet (2 pcs, 18’’ x 18’’ x 0.125’’, National Security Mirror)

LED light strip (3 meters, White, GuoTonG)

Power strip (6-outlet, Belkin)

Backlight shield

Black acrylic sheet (3 pcs, 12’’ x 12’’ x 0.118’’, National Security Mirror)

Fluorescent circline ceiling light (Sunlite, model no. FC12T9/CW) with starter ballast

Zinc threaded rod (4 pcs, 0.25’’ x 12’’, Everbilt)

Hex-plated nuts (24 pcs, 0.25’’, Everbilt)

Temperature and humidity control unit

Heated control module (Coy Lab, serial no. DC1807)

Fan (AC Infinity, model no. LS1225A-X)

Digital humidity controller outlet (Inkbird, model no. IHC200S)

Reptile humidifier (2L, Evergreen)

Beaker (500 mL)

(ii) For swarm plate preparation

Biological material

Enterobacter sp. SM1, SM2, SM3, SM3_18, SM3_24

Reagents

Tryptone (Sigma-Aldrich, cat. no. 91079-40-2)

Yeast extract (accumedia, cat. no. 7184B)

Agar (Research Products International, cat. no. 9002-18-0)

Sodium chloride (Fisher Scientific, cat. no. 7647-14-5)

DI water (0.22 µm filter, Millipore)

Equipment

(iii) Equipment

Labconco Purifier Class II Biosafety Cabinet (Delta Series)

Falcon 14 mL Polystyrene Round-Bottom Tube (17mm x 100 mm, Corning)

Sterilized wood sticks (2.5’’)

Pipet-aid (Drummond, 1000000 µl) with appropriate pipette

New Brunswick Innova 4300 Incubator Shaker

Weighing paper (4’’ x 4’’, Fisher Scientific, Cat. no. 09-898-12B)

Laboratory spatula (4 pcs, 6.5’’, stainless steel, Home Science Tools)

Pyrex glass bottle (250 mL, Corning, model no. 1395)

Pyrex graduated cylinder (500 mL, Corning, model no. 3022)

Hot plate magnetic stirrer (Barnstead International, model no. SP46925) with appropriate magnetic stir bar

Thermo-insulate gloves

Autoclave machine (AMSCO Scientific, model no. SV-120) with autoclavable tray

Petri dish (100 mm x 15 mm, sterile, polystyrene, Fisher Scientific, Cat. no. FB0875712)

Micropipette (0.5 - 10 µl, eppendorf, Cat. no. 3123000020)

Lenovo Yoga 3 Pro laptop computer

Scissors

Acrylic cutter (Fletcher)

Circle cutter (Bott)

Hand drill/table drill with appropriate drill bit set

Hacksaw

(iv) Software

Adobe Photoshop CC 2017

ImageJ 1.52g image analysis software (https://imagej.nih.gov/ij/)

Python based area quantification software (codes available upon request)

Procedure

Part 1, set up the photography incubator

Time: 1 d

i) Assemble the camera stand

Time: 3 h

1. Setup the Yaheetech hydroponic tent according to the instruction manual. The manual comes with the product package. Assemble the skeleton first and then cover it with the polyester material.

2. Use M5 bolts and nuts to assemble the camera frame, as illustrated in figure 1. Connect the Zinc-plated slotted angles first and then the aluminum bars. A rough estimation of the position of the aluminum bars is enough since they are just used to stabilize the structure. The slotted angles have holes by the side, but the aluminum bars don't. Drill holes by the ends of the aluminum bars with hand drill or table drill.

3. Use acrylic cutter to cut the sides of 18'' x 18'' acrylic sheets slightly to fit in the sample platform.

4. Use the circular cutter to cut a circle 9'' in diameter out of one of the 18'' x 18'' acrylic sheets "A" from the center and leave the other one "B" uncut.

5. Fix the sheet "A" on the sample platform. Drill appropriate holes on the side to allow bolts to go through all the way down to the holes of slotted angles.

6. Fix the camera on the camera fixer with its lens facing downwards. Adjust the fixer back and forth to align the camera with the circle on the acrylic sheet.

7. Tape the LED strip around the sample platform 1 inch above the acrylic sheet on the slotted angle. The position of the light cannot be too high because we want to avoid unnecessary reflections of the light from the plates.

8. Cut two pieces of black cloth around 20'' x 20'' in size. Place one of them on the bottom of the tent as black photography background. For the other piece, cut a hole in the center to let the camera lens go through and hang the cloth onto the camera platform to shield any reflection from the top.

9. Place the camera stand inside the tent.

10. Load batteries for the LCD timer remote control and connect it with the camera through the hole on the top of the tent. Tighten the hole using the elastic cord.

ii) Make the light shield.

Time: 3 h

11. Cut circles of diameter 9.2 cm, 5.75'' and 9.5'' respectively out of three pieces of

acrylic sheets. Drill ¼’’ holes on each of the corners of the sheets 3 cm from both edges. Assemble the light shield according to figure 2. Place the circular fluorescent light bulb between level I and the sample platform. Stick the starter ballast under the sample platform using tapes.

12. (Optional) Sometimes, the light bulb comes with metal spring clamps. You can fix the clamps on the sample platform and clamp the bulb.

Pause point: There are two modes of illumination. One is using backlight and the other is using side light (Figure 3). When taking high quality image of one-plate event, the light shield is used, and it has its own backlight light source. To take photos, place the light shield on the sample platform and align it with the camera. Turn off the LED light when using the light shield. On the other hand, when taking photos for more than one plate. The light shield is removed and the uncut 18’’ x 18’’ acrylic sheet is put on top of the circularly cut sample platform. To take photos, turn on the LED light as side light source.

iii) Install the temperature and humidity control system

Time: 2 h

13. Put the heat control module inside the tent on the side of the bottom so that it will not show up in the swarming photos when using the light shield. Adjust the temperature setting to the desired temperature for swarming assay.

14. Set the humidifier outside the tent and connect the power cord to the humidity controller outlet. Extend the extractable plastic mist tube through the hole on the tent wall into the tent beneath the sample platform. Notice: check the camera preview and make sure the tube and the mist do not show up in the image.

15. Fill the humidifier tank with water. Plug in the humidity controller to the power strip and adjust the humidity value with tolerance range according to the controller manual. For *Enterobacter* sp. SM3, set the humidity for 40% RH ± 5%RH tolerance (tol).

16. Place a 500 ml beaker under the mist tube to collect water droplets from the mist tube.

17. Fix the AC fan on one of the slotted angle legs facing the beaker using the bolts and nuts that come with the fan. The fan is used not only to blow the mist from the humidifier to avoid the fog

showing up in the photos but also to improve ventilation and uniformity of the temperature and humidity in the chamber.

18. Tighten all the holes and seal the zip of the tent.

Part 2, perform swarming assay

Time: 2 d

i) Grow bacteria suspension

Time: 16 h

19. Weigh 0.5 g yeast extract, 1 g tryptone, and 0.5 g NaCl, placed in a 100 mL Pyrex bottle. Measure 100 mL deionized water and pour in the bottle.

20. Close the lid and shake the bottle until all the powders dissolve in water. You should see a clear yellow solution at this point. The solution is called LB broth (Lysogeny Broth).

21. Loose the bottle lid and put in an autoclavable tray with a thin layer of water inside. Autoclave the LB broth for 25 minutes under 15 psi pressure and 121 °C.

22. After autoclave is finished, take out the tray and wait for the broth to cool down to room temperature. Transfer 5 mL LB broth to a 14 mL tube using pipet aid in the hood.

23. Bring out the bacteria glycerol stock out of the -80 °C freezer. Use a piece of sterilized wood stick to scratch the bacterial containing ice surface and then dipping the stick into the LB broth. Put the glycerol stock back to the freezer and shake the inoculated LB broth overnight (~16 h) in the shaker. The temperature is set to 37 °C for SM3 and shaking frequency 200 rpm (revolutions per minute). Prepare the overnight suspension around 5 pm so that it will be ready for use around 9 am the next morning.

ii) Prepare the swarm plates

Time: 5 h

24. Weigh 0.5 g yeast extract, 1.0 g Tryptone, 0.5 g NaCl, 0.5 g Agar and put them in a 100 mL Pyrex bottle. Measure 100 mL deionized water and pour it in the bottle.

25. Put the magnetic stir bar of appropriate size in the bottle, loosely close the lid, and place the bottle on a hot plate magnetic stirrer. Turn on the heat and the magnetic stirrer. It takes roughly 10 -

15 minutes for the medium to boil.

CAUTION: DO NOT LEAVE THE HOT PLATE UNATTENDED.

26. Turn off the stirring and heating after the medium start boiling. Wear thermos-insulate gloves to transfer the bottle to an autoclavable tray with a thin layer of water.

27. Loosen the bottle lid and start autoclaving for 25 minutes under 15 psi, 121 °C.

28. Once the autoclave is done, put the bottle of medium back to the magnetic stirrer with heating function off but stirring function on. In this step, we want the medium to cool down to 40~50 °C and the constant stirring is to avoid non-uniformity in the agar.

29. Once the target temperature is reached, set a flame and use a pipet aid to transfer LB agar medium to 6 petri dishes, with 15 ml on each petri dish.

30. Turn off the flame and wait for the agar to solidify. This will take about 2 hours.

31. (Optional) Use the freshly made plate or store the swarming plates in 4 °C cold room with them inverted for up to 2 days. Whenever you plan to use the swarming plates, you need to dry the plates in the hood first. This is to remove water on the agar surface. If there’s water on the agar surface, the bacterial motility may be swimming rather than swarming. Thus, the next step is crucial.

32. Remove the lid of swarming plates in the hood. When the room humidity is above 50%RH, dry the plates for 20 minutes. When the room humidity is below 30%RH, the drying time is about 10 minutes. When the humidity is between 30-50%, you can adjust the drying time accordingly to around 15 minutes. Do not over dry the plates; otherwise, bacterial cells may not be able to swarm, due to either surface friction or dryness.

33. Use micropipette to inoculate 2 µL overnight bacteria suspension on the center of a swarm plate. Transfer the swarm plates into the incubator after the inoculation drop dried (3D hemisphere turns to a 2D circle).

Part 3, time-lapse photo taken and swarming rate quantification

Time: 1 d

34. To use the light shield, put one swarm plate inverted on the plate holder. Turn on the fluorescent light bulb and the camera. LED light strip stays off.

35. In the preview of the camera, you should see the plate sitting in the center of the screen. Otherwise move around the light shield to align the camera with the sample.

36. Rotate the nuts on the threaded rod to adjust the position of the acrylic sheets. The distance between the sample platform and Level I is about 1.5 inches. The distance between Level I and Level II is slightly under 1 inch (about 2 cm) while Level II and Level III are separated by 3.5 inches. If you see a round light spot on the petri dish, slightly lift Level III. If the petri dish is too dark, slightly lower Level III. If you cannot get a good image by adjusting Level III, then adjust Level II slightly up or down. **Caution**: Calibration of the light shield takes practice. There is a subtle distance relationship between each sheet to achieve the best image quality depending on the camera setting. We want the light to shine through the transparent agar and be reflected from the swarm colony. Once you find the right position, tighten all the nuts and the position is locked for later imaging.

37. Set the camera focal length to 35 mm - 65 mm. Adjust the zoom ring to have the sample occupy the full screen but not exceed the border. Use “M” manual mode to focus on the bacteria colony. The aperture is set to F5.6 -F7.1 and adjust the shutter speed until the resultant exposure value is 0 or -⅓. This is to make image processing easier because overexposed images will lose information details.

38. For multi-plate assay, remove the light shield, turn on the LED light strip, and place the uncut acrylic sheet on the sample platform. Place the swarm plates inverted on the acrylic sheet so that water will not condensate on the lid. Check the camera preview to make sure all the plates are within the range of the screen.

39. (Optional) The mode of the scene can be “Auto” or “fluorescent bulb”. If you find the image too yellowish, you can adjust the white balance to add more blue tone. You can save the images as raw format if the memory card space allows.

40. Set the frame rate and frame number of the LCD camera timer control according to the manual. In the case of SM3, we set the frame number to 50 and time interval between frames as 15 min. Press “start” button to start time-lapse photo shooting.

41. Seal the zip of the tent. The swarming assay may take about 10 h. You can leave the camera on for overnight and collect the images the next morning. During the photo taking process, DO NOT

shake the incubator; otherwise the optical setup may be perturbed.

42. Make sure there is enough water in the humidifier water tank. If you want to check the condition inside the chamber, you may gently open the window on the top of tent to see the camera screen without disturbing the photographing.

43. Stop the timer controller when the swarming is finished. Connect the camera with a laptop using a USB data wire and download the images to the designated folder. Detach the laptop from the camera.

44. Check the images taken in your computer. If the brightness of the images varies, you can use the “Stack Deflicker” plugin in ImageJ to calibrate brightness. If the sample position in the images varies over time, you can apply the “Image Stabilizer” plugin to fix it.

45. (Optional) Use a common software (Photoshop CC, ImageJ, etc.) to render a time lapse video of the images. Or you can just use ImageJ. Go to “File” -- “Import” -- “Image Sequence” to Import the images. Go to “Image” -- “Stacks” -- “Animations” -- “Animation options” to set the frame rate you want. Then, save the image sequence as an AVI file.

46. To manually calculate the swarm area, drag the image that you are interested in to the ImageJ control bar. Go to “Analyze” -- “Set Scale” to calibrate the ratio of pixels to actual length. Then use the freehand “selections tool” to outline the colony edge and press “M” to calculate the area of the selected colony image by image.

47. To calculate the swarming speed automatically, open the python script and change the file directory to the directory where you store your image sequences. Running the script will calculate the swarming colony area over time and save the data in a .txt file. The python script utilizes the subtraction edge detection algorithm to identify the edge of the swarming colony and then calculate the enclosed area value in each frame uploaded.

Troubleshooting

Problem 1: I can’t get 18’’ slotted angles and it is hard to cut a circle out of a piece of acrylic.

Reason & solution: Sometimes certain model of products will be out of stock. In this case, one can try to cut the 36’’ into two halves with a hacksaw. To cut the circle, if one plans to use a hole saw or circle cutter, make sure to clamp the acrylic sheet first. One alternative is to go to an engineering

workshop or carpenter's shop. They have the saber saw or jigsaw to cut the circle.

Problem 2: Swarming strain does not swarm. They just form a dense colony spot by growth.

Reason & solution: The reason for this is due to roughness of the agar surface or the plate is too dry. Always use fresh plates or two days old at most. Beyond two days, the agar plate will become significantly drier than before and may inhibit swarming. Drying time in the hood may be too long. When the lab humidity is below 30% RH, 10 min of drying is enough. Also, pour the plates when the agar solution is not too cold. Cold agar will result in rough surface since solidification has already taken place in the bottle, forming small clusters. Finally, double check the tryptone or yeast. Occasionally, for certain Lot numbers, the chemicals do not dissolve thoroughly, which may change the texture of the agar surface and increase surface friction.

Problem 3: Non-swarmer strains also "swarm", even faster than the swarming strains.

Reason & solution: The agar concentration may be too low, or the drying time too short so the cells are swimming not swarming on the plates. Different swarming species may have different agar concentration tolerance. For SM strains, 0.5% agar is the concentration to distinguish swarmers and non-swarmers while for *B. Subtilis* 3610, 0.7% agar is the concentration to tell non-swarmer *B. Subtilis* DS215 from the swarming wild type. To fix the problem, one can try to increase the drying time, pour the plate when agar solution is colder, or raise the agar concentration.

Problem 4: The swarming rate of the same strain varies a lot among different trials.

Reason & solution: The swarming rate may vary under different optical density (OD_{600}). For SM bacteria strains, when the cells are at the first half of the growth exponential phase, or the death phase, the swarming lag is shortened and variations in swarming rate and morphology was noticed. However, in stationary phase (OD_{600}: 1.5~1.8), the swarming rate hardly fluctuates. For, different bacteria species, situation may differ, but our suggestion is to use stationary phase cells every time one performs the assay.

Problem 5: Condensation still happens, what shall I do?

Reason & solution: If one uses another thermo-insulate box instead of the one mentioned in the

protocol, or uses another kind of temperature control unit, this could happen due to temperature gradient. Try to set up the plates upright instead of inverted, the condensation problem may be resolved.

Time Taken

Step 1-10, camera stand assembly: ~3 h.

Step 11-12, light shield making: ~3 h.

Step 13-18, environment control system installation: ~2 h.

Step 19-23, overnight bacteria culture growth: ~16 h.

Step 24-33, swarming plates preparation: ~5 h.

Step 34-42, incubation and photo taking: ~10 h (it may vary among different bacterial species).

Step 43-47, image processing and swarming area quantification: ~3 h.

Overall, it may take one week to complete the full protocol.

Anticipated Results

1. After step 18, the incubator is complete. The humidity and temperature should reach the setting value within 10 min after the power is on.

2. After step 42, photos of the swarming assays are taken, and one can use either ImageJ or Photoshop CC to render a swarming movie as shown in Supplementary video 1 & 2.

3. After step 47, the area of swarming colony at each frame is calculated. The relation between swarming area and time can be plotted as shown in figure 4.

Supplementary video 1 ---- **Time lapse video of *Enterobacter* sp. swarming assay taken with the light shield.** 5 µL overnight culture of SM1, SM2, and SM3 were inoculated on 0.5% LB agar plate and incubated in the swarming incubator. Time lapse images were taken for 10 hours and the images were rendered into a .mp4 video file using Adobe Photoshop CC 2017 at 11 frames per second (fps).

Supplementary video 2 ---- **Time lapse video of *Enterobacter* sp. SM3 swarming assay taken with LED light.** 2 µL SM3 and its mutant (SM3_18 or SM3_24, each in triplicates) overnight culture were inoculated on 0.5% LB agar plate and incubated in the photo chamber. Time lapse images were

taken for 10 hours and the images were rendered into a .avi video file using ImageJ (ver. 1.59g) at 11 frames per second (fps).

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Figures

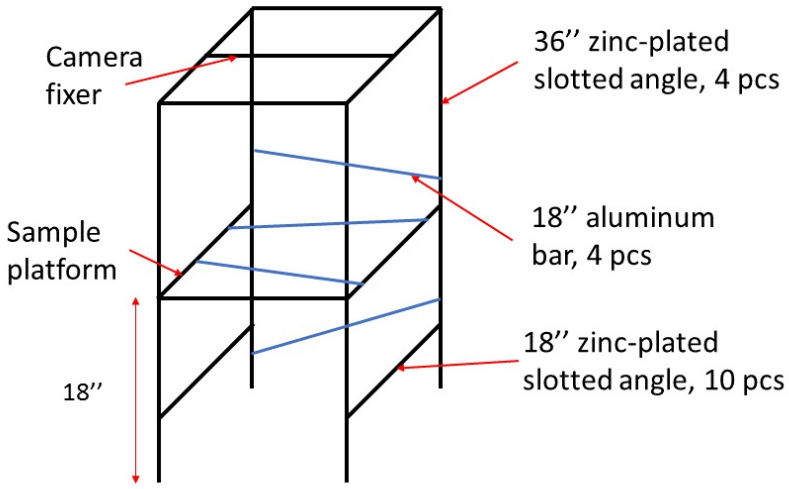

Figure 1

Schematics showing the structure of the camera frame. 4 pieces of 36’’ zinc-plated slotted angle stand perpendicular to the ground and connected by 10 pieces of 18’’ zinc-plated slotted angle using M5 bolts and nuts. The aluminum bars are to stabilize the whole structure with no specific position requirement. When using the light shield, the distance between the ground and the sample platform is 18’’. However, when using LED light illumination, the sample platform can be elevated by several inches to fit the petri dishes there.

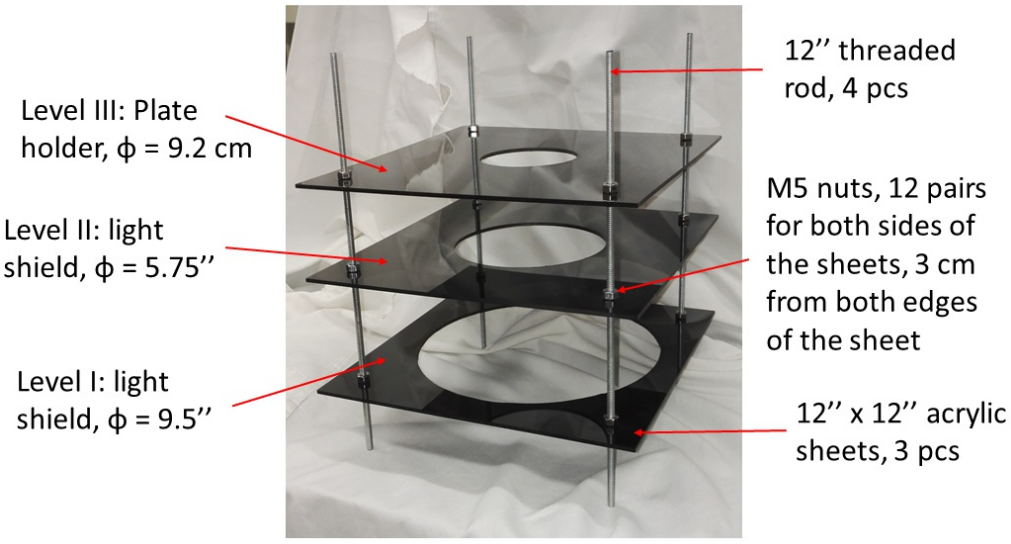

Figure 2

Picture showing the structure of the light shield. 3 pieces of 12''×12'' hollow acrylic sheets were connected by 4 pieces of 12'' zinc threaded rod and fixed using hex-plated nuts. The distance between Level I sheet and the ground is about 1.5'' which allows the circular fluorescent bulb to fit in. The height of Level II and Level III can be adjusted to realize the best illumination.

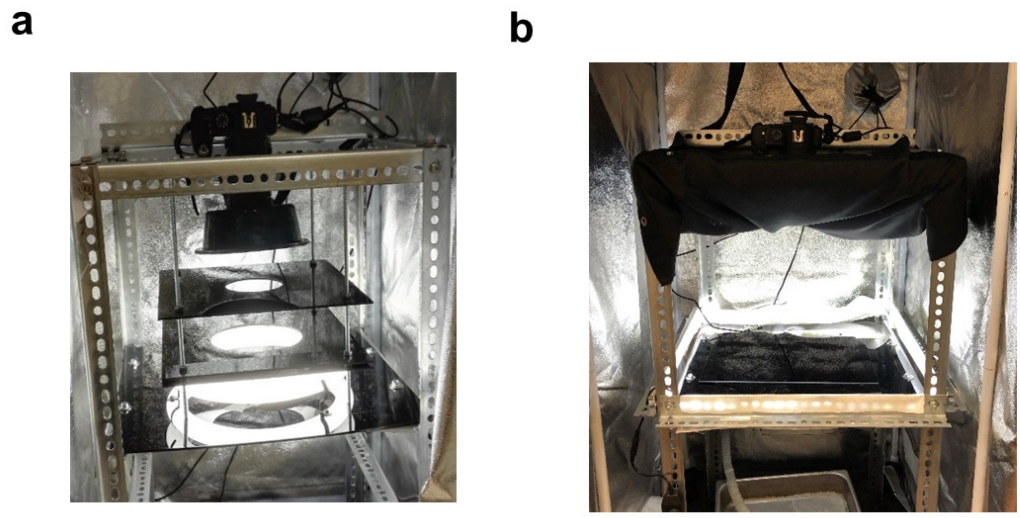

Figure 3

Taking photos using the light shield or LED light strip. a, Taking photos using the light shield. The circular fluorescent bulb is placed between Level I and the sample platform. The distance between Level II and Level I is about 2 cm while the distance between Level II and Level III is 3.5’’. b, Taking photos using the LED light strip. The LED light is fixed 1 inch from the sample platform on the horizontal zinc-plated slotted angles. If the light is too strong, one can insert a white paper belt to diffuse and reflect the light. The black cloth near the camera is necessary for blocking reflections from the plates.

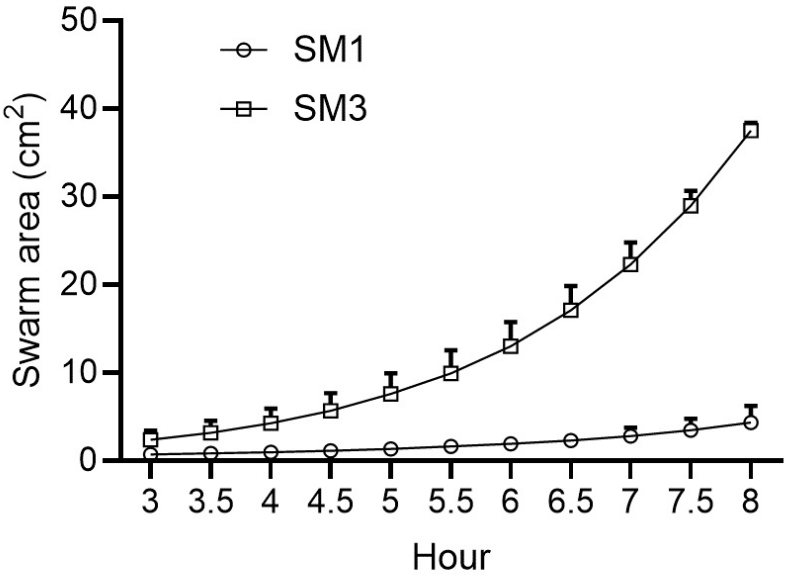

Figure 4

Quantification of Enterobacter sp. SM1 and SM3 swarming motility. 5 µL overnight bacterial culture of SM1 and SM3 were inoculated on 0.5% LB agar plates and incubated in the swarming incubator (repeated 10 times). Time lapse images were taken every half an hour, and the swarm area was measured for each image. Data are represented as means with 95% confidence interval. When not shown, the errors are smaller than the size of the symbols.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Supplementary Video 1.mp4
Supplementary Video 2.avi