Chlamydomonas phototaxis, photoshock and motility

Chlamydomonas reinhardtii is a unicellular, bi-ciliated green alga, approximately 10 microns in diameter, that lives in soil. Like many other organism, it moves in an oriented fashion in the presence of light. This oriented motion is called phototaxis and can be positive (towards light) or negative (away from light). Whether a culture is positively or negatively phototactic, depends on the growth phase, light intensity, previous light exposure, genotype and other completely uncharacterized growth conditions (*Chlamydomonas source book*). The finely tuned photosynthetic nature of the organism is derived from its rhodopsin based eyespot that allows it to detect and move towards light sources and optimize light absorption.

Phototaxis:

Strains:

CC-124 *wild type mt* $^{-}$

CC-125 *wild type mt* $^{+}$
CC-2894 ptx1, This is the best-Characterized non-phototactic mutant.

The two strains, CC-125 and CC-124 are originated from a culture collected from a potato field in Amherst Mass, in 1945.

You have been provided with an upright microscope, *Chlamydomonas* strains, microscope slides, cover glass and 3 LED's (green, red and blue) connected to a power source (battery).

To start, place a small drop (20 ul) of cell suspension on a clean microscope slide, and observe the cells under various magnifications. In lower magnifications, the cells will appear as very small dots, but as you increase the magnification, you should be able to detect the eyespot and the two flagella. How do you characterize the motion of the cells? Do they move randomly or in a directed manner? What is the color of the cells and the eyespot? Where is the eyespot located, with respect to the cell body? Can you approximate the size of the eyespot? We are using non-treated glass, and about 10-20% of the cell with stick to the glass. Find a stuck cell and observe it with high magnification. Can you see the two flagella?

Now connect the green LED to the power source and bring it close to one edge of the drop as you observe the behavior of the cells under microscope. What do you observe? Can you see the difference in the cell motility, in the presence and absence of the green light? Move the slide so that you can observe the droplet edge closer to the LED and further away from the LED, do you see a difference in number of cells near the two edges? Now move the green LED to the other side of the droplet and observe the motion. What do you see? Repeat the experiments with different color LED's and different strains and observe the differences between the phototactic behavior.

Photoshock:

The cells will show another light induces reversal of swimming direction, known as photoshock. In contrast to phototaxis, the photoshock response does not appear to depend on directional cues, so its photoreceptor could be located anywhere in the cell. However, it is usually assumed that the eyespot is also the site of light transduction for photoshock.

To observe the photoshock response, increase the light intensity of your microscope to maximum and cover the light source with the red filter provided. Observe the motion of the cells for few minutes in the red light. After about 2-3 minutes, suddenly remove the red filter and observe the cell motion. Do you see a qualitative difference between the random motility and the photoshock response?

Motility:

While observation of the axonemal ultra-structure requires a high-resolution bio-imaging technique (e.g. electron microscopy), many important characteristics, such as number and length of flagella, swimming behavior and velocity, can be observed under a light microscope. The following strains are provided to you:

Wild type (CC-125 WT+):
This is the "normal" *Chlamydomonas* strain (same as for the phototaxis part). It has two flagella; each is ~11 µm long. The wild type swims with ~50 Hz (50 beats per second) and a speed of ~140 µm/s. Without a high speed camera, you will not be able to see the flagella in freely moving cells, but you will be able to follow the movement of the cell body. Some flagella will stick to the glass slide, in which case you will be able to observe these as well.

pf14 (paralyzed flagellum; CC-1032+)
This mutant strain has a defect in a single gene (*rsp3*), which prevents assembly of the radial spokes, a multi-protein complex required for flagellar motility. Cells of this mutant strain are able to build two regular length flagella, which can occasionally "twitch" but fail to produce effective movement. As the flagella are not beating in this strain, we can clearly view them.

pf3 (CC-1026+)
This strain has defects in the Nexin-Dynein Regulatory Complex, a multi protein complex that regulates dynein motor proteins and connects neighboring doublet microtubules via nexin links. These defects result in a reduced swimming speed (~ only 1/3 of the wild type swimming speed).

mbo1 (move backwards only; CC-2679+)
This mutant shows no regular forward swimming, but instead a slower backwards swimming motion. Because of the low speed many of these cells will get stuck on the glass slide, but some cells that move backwards should be visible.

uni1 (CC-1926+)
Cells of this mutant strain have only one flagellum (instead of the regular two in the wild type), display no directed forward swimming motion and spin in circles instead.

vfl2 (variable flagella; CC-2530+)
This mutant produces cells with variable numbers of flagella ranging from zero to six. Depending on the number of flagella swimming movement varies considerably and includes paralysis and spinning.

Additional solutions:

TAP medium (Tris-Acetate-Phosphate medium):
This is the medium in which all strains were grown. If cell concentrations of some of the strains are too high, you can use the TAP medium to dilute your samples.

10% Ficoll (in TAP medium):
Ficoll is a high molecular weight polymer that changes the viscosity of the medium affecting waveform, beat frequency and swimming speed. You can observe changes in swimming speed by mixing for example equal parts of the wild type strain and Ficoll solution and compare this side-by-side with the wild type strain without Ficoll added.

Salmonella Large-Scale Collective Motion

Salmonella is a genus of rod-shaped, gram-negative, non-spore-forming bacteria with diameters around 0.7 to 1.5 μm, lengths from 2 to 5 μm, and several flagella that it uses to propel itself through fluids. *Salmonella* is closely related to *E. coli* and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. Some strains can be pathogenic if ingested, and as a result *Salmonella* is synonymous with food poisoning in popular culture. However most strains, including the laboratory strain we are using today, are harmless and require no special handling. Nonetheless, it doesn't hurt to exercise caution by wearing gloves and washing hands after the experiment.

Like most low-Reynolds number swimmers, *Salmonella* uses flagella to locomote through fluids. The particular way in which *Salmonella* propels itself, e.g., using its flagella to push fluid away from its body, puts it into a class of swimmers termed "pushers" which generate a far-field hydrodynamic flow described by a positive force dipole wherein fluid is pushed away from the body along the swimming axis while drawn in along the sides. In contrast, *Chlamydomonas* propel themselves by using a breaststroke-like flagellar motion and as a result generate an attractive flow field along the swimming axis and repulsive flow along the sides, consistent with a negative force dipole. Such organisms are termed "pullers".

There is a striking difference in the large-scale flow behavior of highly concentrated suspensions of pushers vs. pullers. Microscopically, hydrodynamic interactions between two pushers tend to align their swimming directions, and in dense suspensions of pushers, macroscopic instabilities emerge in the form of large-scale coherent jets and swirls with length scales 10-100 times the size of an individual cell. The opposite is true for pullers, i.e. microscopic hydrodynamic interactions tend to favor anti-alignment with reorientation away from each other, frustrating large-scale coherent motion. The goal of this lab is to observe this large-scale collective motion by looking at concentrated *Salmonella* suspensions.

Experiment:

Strains:

SJW-1103 *helical flagella strain*

You have been provided with an upright microscope, microscope slides, 2 Eppendorf tubes each containing 1.0 mL of log-phase *Salmonella* in LB broth, beaker for waste, and access to a tabletop microcentrifuge.

To start, place a small drop (~20 ul) of cell suspension on a clean microscope slide, and find the cells under higher (> 40 um) magnifications. Be careful not to dip the objective into drop. Ask for help from one of the TA's if you are having trouble seeing the cells. You should see cells executing run-and-tumble behavior, i.e., ballistic motion punctuated by periods of random tumbling in which their direction of motion is randomized. Do they move independently or is there collective motion?

Next, concentrate the cells by centrifugation. Load both tubes into diametrically opposite wells in the centrifuge, close the lid, and spin down for 10 minutes at 5,000 rpm. After the spin is done, carefully remove the tubes from the centrifuge. You should see a pellet of bacteria at the bottom side edge of both tubes. For one of the tubes carefully pipette out about 950 uL of the supernatant, taking care not to disturb the pellet, and pipette it into the waste beaker, leaving ~ 50 uL in the tube. Resuspend the pellet by tapping on the side of the tube with the cap on. When the pellet is resuspended, the fluid should have a cloudy appearance.

Take out ~ 20 uL of the resuspended dense suspension as before and place it on a clean microscope slide. Using the same magnification as before, focus on the drop until you can see the bacteria. Again, be careful not to touch the objective to the drop. How would you describe the motion of the dense suspension of bacteria? Does it resemble anything at all like the run-and-tumble motion of the individual cells? What is a typical length scale of the motion and how does it compare to the size of an individual bacterium observed previously? Finally, how does the motion at the edge of the drop differ from the middle?

The instabilities that you are (hopefully) observing are a result of an interplay between intercellular hydrodynamic interactions, bacterial aerotaxis (the directed motion toward oxygen at the liquid-vapor interface of the droplet surface), and gravity. Besides being mesmerizing to observe, it is believed that such convective instability patterns can benefit the population as a whole by promoting the distributing oxygen and nutrients into the bulk of the droplet, where it is most needed.