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Flagello Shock

Chlamydomonas reinhardtii are green algae, with two flagella, but these are not essential organelles for survival. They have an utility during reproduction, by allowing movement and attaching themselves with their flagella to other *Chlamydomonas* of compatible mat-type. *Chlamydomonas* are used to lose their flagella before dividing and during mating.

Exposed to a stressful environment, Chlamydomonas detach their flagella, presumably to reduce their surface of exposition to harsh conditions. With the same idea, we assumed that losing flagella in stressful environments was selected through evolution: Disabling *Chlamydomonas* reproduction could avoid compromised offspring due to protein denaturation or higher rate of errors in DNA replication when harsh conditions appear. Reintroducing a normal environment for *Chlamydomonas* leads to a flagellar regeneration, thus testifying of their utility.

We found a protocol explaining how to observe flagellar regeneration. *Chlamydomonas* usually live in an environment of pH 7. When they are put in an acid medium, the pH shock will induce a chemical deflagellation. Around 1 minute after, the *Chlamydomonas* are reintroduced in a medium of pH 7 and you can start to observe and measure flagellar regeneration by fixing a sample in Lugol solution for later microscopy observation (x400 magnification). Observation is made every 15 minutes to see the progress in the regeneration over time.

We decided to adapt the initial protocol in order to answer our following question :

What is the flagellar regeneration of *Chlamydomonas reinhardtii* according to pH ?

or

At what pH can *Chlamydomonas* keep their flagellum ?

Protocol :

To occur the deflagellation, we do the pH shock by using acetic acid : in a solution of , we add acetic acid (0,5N) to decrease the pH. In this step, we will add different quantity of acetic acid in order to test different pH environments to test the ability of to keep their flagella.

We tested 3 different pH of deflagellation :

- pH 7 as our negative control, no deflagellation should be observed.
- pH 5.5 is our experimental pH.
- pH 4 as our positive control, all Chlamydomonas should be deflagellated according to literature

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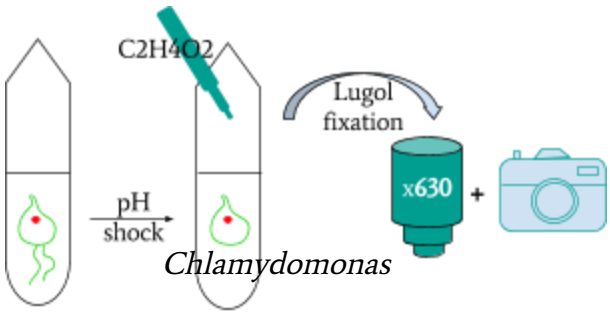

Schematic of our experiment

Sample size : 3 solutions
Repetitions : 3 slides for each sample
Replicates : 3
Details : For 20µL of solution, you need 1µL of Lugol to fix the solution.

Materials:

- Chlamydomonas solution (420µL) x3
- Acetic acid (0.5 M concentration)
- Lugol solution (1µL) x2
- pH paper
- Microscope slides x3
- Microscope cover slip x3
- Fluorescence Microscope x630 magnification
- 4 pipettes (5 µL, 10µL, 20µL and 25µL)

Preparation :

1. With pH paper, control the pH of 10µL of the solution and the acetic acid solution.
2. Fix 10µL of the solution in one of the Lugol solutions.
3. Take 5µl of the fixed solution and put it on a slide in order to observe it under the microscope in order to see if the are flagellated .

Experiment :

1. Add to the solution :
   a. 0 µL of acetic acid → Negative control
   b. 10 µL of acetic acid
   c. 25 µL of acetic acid → Positive control
2. Measure the pH of the solution with 10 µL of it and a piece of pH paper.
3. After 1 minute of pH shock, fix 20 µL of the solution in 1 µL of Lugol solution.
4. Take 5µl of the fixed solution and put it on a slide in order to observe it under the microscope (repeat this step for 3 different slides for repetitions).
5. Count the number of with and without flagella.