Lab Notebook

Interaction between phototropism and geotropism in *Phycomyces blakesleeanus*

Summary

6/2/2017

Finding & preparing a media

We've decided to use **PDA** (Potato Dextrose Agar). The receipt we used was:

- 1000 ml of distilled water
- 200g of sliced potatoes (washed but still with the peel)
- 20g of dextrose
- 20g of agar powder

Method:

- Boil the sliced potatoes with the 1000 ml of osmotic water for 30 minutes. If you boil them for more than 30 minutes, the water will evaporate and the potatoes will burn.
- Once the potatoes boiled, filter the liquid with a filter (we used a coffee filter). To be sure that the filtered liquid contains starch, we test with 1 MxL^{-1} KI. If the solution gets blue, it means that it has starch.
- When the liquid is completely filtered, add enough osmotic water to reach a liter of solution.
- Add 20g of dextrose to the solution and then 20g of agar.
- Autoclave for 15 minutes at 15 psi (121°C).
- Once autoclaved, distribute equally in the plates. We put 25mL in each plate, we pour the PDA media on 29 plates.
- Let them cool down for 10 minutes, and parafilm them before taking them out of the hood.

Testing

Today we have also done some tests to know how to **inoculate** our *P. blakesleeanus*. After some researches we found that it was mostly made out of a **spore** solution. So we thought that we could isolate a spore from the cultive we had and inoculate it in a YPD plate (the ones we had in the lab).

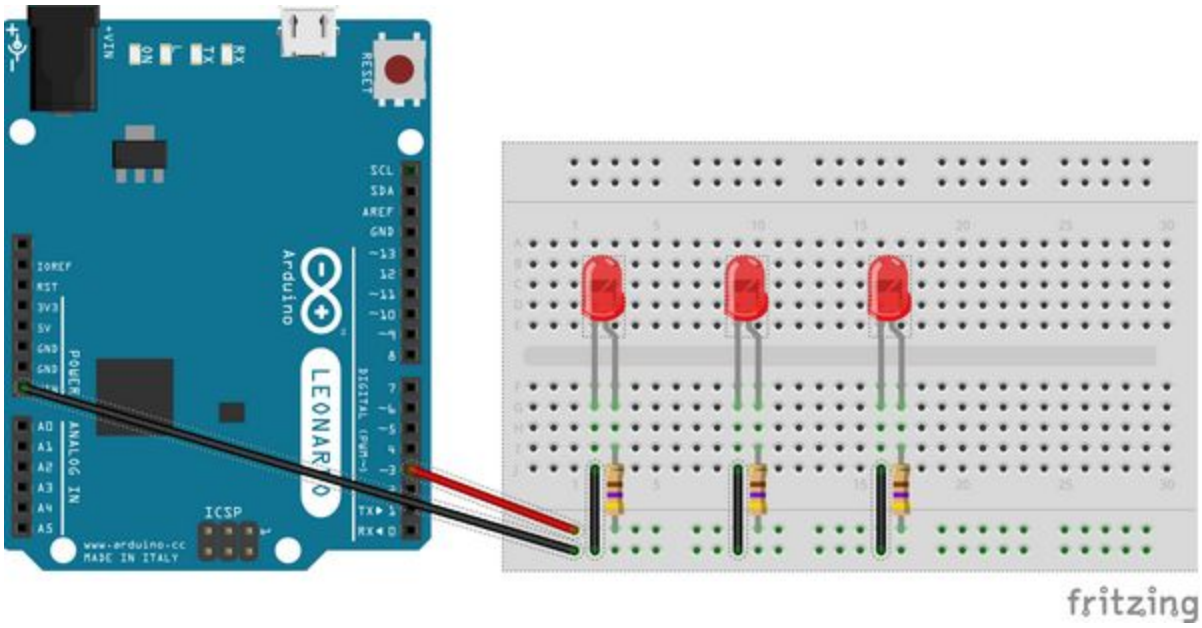
Isolate a spore was difficult, so we tried to do three other tests (about **10h30**):

- Put a hypha in the middle of the plate
- Rub the loop in the PDA of our culture and then streak it in the middle of the Petri Dish
- Rub the loop in the PDA of our culture and then streak it as usual.

At **18h02**, we still can not see any growth on our Zygomycetes; that has been kept at 20°C with full light. We've switched off the light.

Arduino setup

We've also done the **Arduino setup**:



Using this code to light up the LEDs at **different intensities** :

```
//Constants, here each Pin which will be used
const int ledPin1 = 5;
const int ledPin2 = 10;
const int ledPin3 = 3;
const int ledPin4 = 6;
const int ledPin5 = 9;

//Give the intensities for each LED : 64 = 25%, 127 = 50%, 191 = 75%, 255 = 100%
int val1 = 255; //100%
int val2 = 51; //20%
int val3 = 102; //40%
int val4 = 153; //60%
int val5 = 204; //80%

void setup() {
  // Initialise each Pin as an output with pinMode()
  pinMode(ledPin1, OUTPUT);
  pinMode(ledPin2, OUTPUT);
  pinMode(ledPin2, OUTPUT);
}

void loop() { // Give to each Pin a value, here an intensity
  analogWrite(ledPin1, val1);
  analogWrite(ledPin2, val2);
  analogWrite(ledPin3, val3);
  analogWrite(ledPin4, val4);
  analogWrite(ledPin5, val5);
}
```

On out setup, as you can see in the code, we connected the different set of 3 LEDs at different pins (3, 5, 6, 9 and 10).

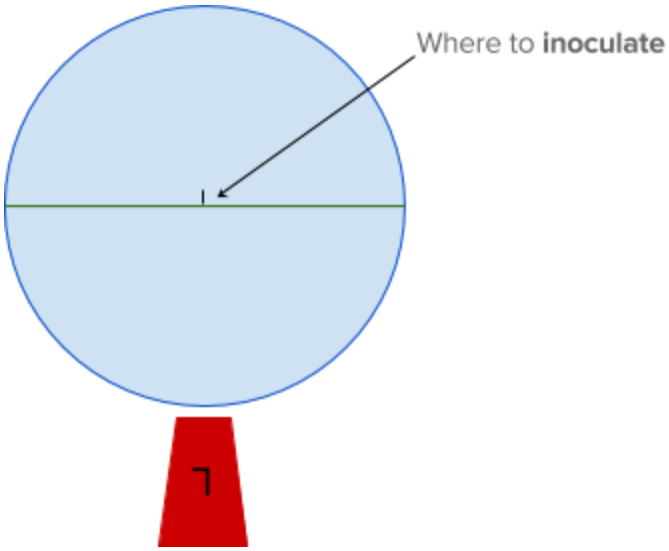
Controls were done with a second arduino. The code of this one only took in account the 100% intensity.

7/2/2017

Preparing the culture

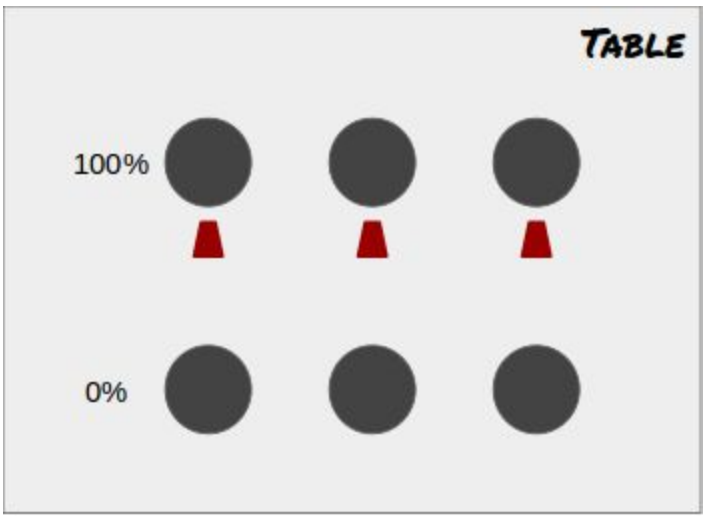
Label each plate with your name, the date, the media, and the organism you will inoculate in the plate.

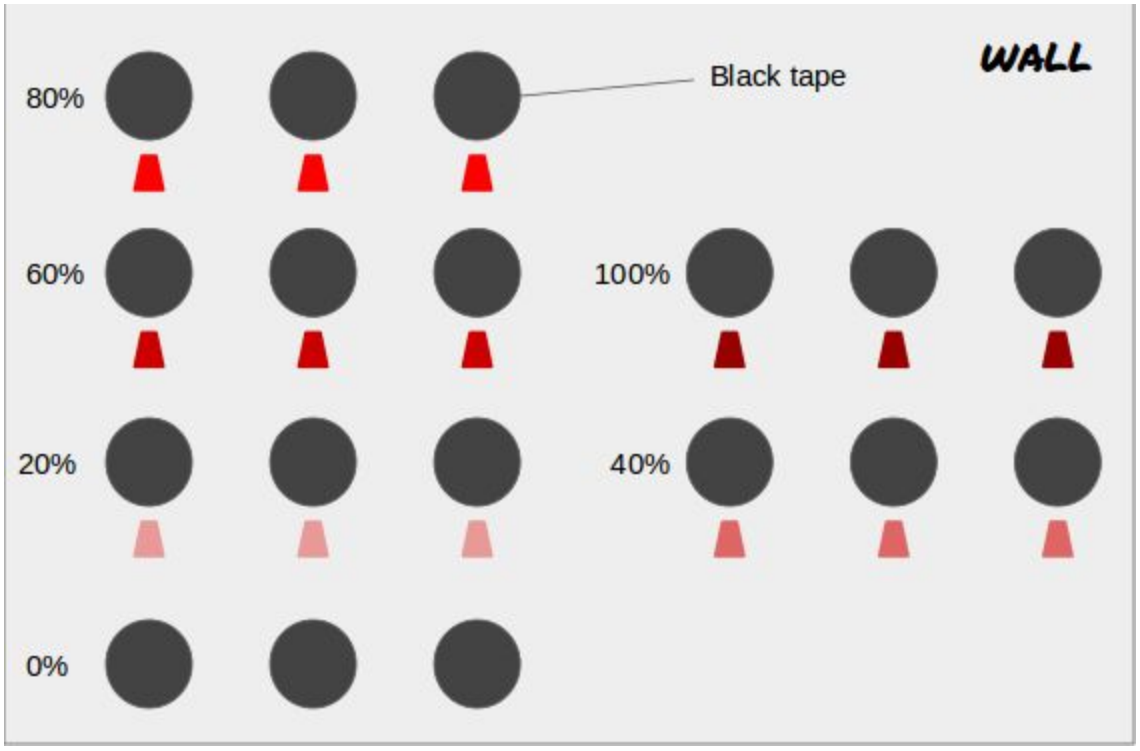
Draw a **line** in the middle of the plate (like the diameter of it), and in a middle of the line (or the center of the plate) draw a **small mark**. This is where the organism will be placed.



Under **sterile** conditions, thanks to an electric Bunsen burner, inoculate the culture on each plate in this way :

- Light up the Bunsen burner
- Manipulate in the sterile zone it creates
- With a loop, **rub the media** in the tube and then, rub the loop on the mark in the plate.
- **Parafilm** the plate again
- Put black tape on the top side of the plate, to prevent light from entering.
- Because tape doesn't let O_2 enter, **pierce** it with a needle -below the cover of the plate-.
- Scotch the plate on the wall (as shown in the drawing), with a lot of tape otherwise the plate will fall.





Phycomyces blakesleeanus

Our cultures have grown a little bit. We can see them well in the **microscope**. We have two organisms growing in the completely streaked plated (at the beginning of the streaking). On the plate where we put a hyphe; we had about six new organisms growing next to it that we could see also on the microscope.

We've asked **Luis Corrochano** (who works on *Phycomycetes* genetics) about the **growing** of this organism and how did we inoculate them. He said that *Phycomycetes* takes several days to grow, but that we would have a *Phycomycetes* with mycelium and sporangiophores on **4-7 days**. We won't have time this week to see this growing, but we will be able to, at least, see the organisms and their development on the microscope.

8/2/2017

Plates fell!

This morning we got up with plenty of our plates in the floor. The ones that **fell** were:

- **0%:** 1, 2 and 3
- **20%:** 2 and 3
- **40%:** 1, 2 and 3
- **80%:** 2 and 3
- **100%:** 1 and 2

Because *Phycomyces* didn't grow this night (we could not see them without a microscope), we decided to **retape** the plates on the wall. With more much more tape, to be sure they won't fall. We also taped the ones that didn't fall.

Data analysis

We've also started thinking about how to analyze our data.

To quantify: Fungi's area.

Measure the area below and above the line that we did on the plates (that we situated parallel to the floor).

What do we attend to see: How many fungus did grow up (negative geotropism) and which did grow down (phototropism).

Compare: Knowing that A is the area above the line and B the area below, we decided to do the next calcule to compare them:

Where f will be the **percentage** of area above the line in relation to the total area. This will normalize all the comparaisons.

On our code we've also added the condition in which we won't have any area above the line (taking in account that the **.csv** will have a line for the area above and the next one for the area below):

```
import numpy as np
import matplotlib.pyplot as plt
```

```
import matplotlib
from math import *
import csv

# Execute this program with Python2

Inten = [0, 0, 0, 20, 20, 20, 40, 40, 40, 60, 60, 60, 80, 80, 80, 100, 100, 100]
allratio = []

def donnee(file):

    a = []
    with open(file, 'rb') as csvfile: # Open the file
         reader = csv.reader(csvfile)
         reader.next()               # Switch the first line
         for row in reader:
                b=row[1]                    # Take the second row
                a.append(float(b))          # Converts itin a float
        for i in range(0,6,2):       # Iterates on the pair numbers between 0 and 6: 0, 2, 4
               if i < 1:                    # If there's no area above or below the line
               p = 0                 # make the ratio equal to zero
               else:
               p = a[i]/(a[i+1]+a[i])  # make a pourcentage of the total area that is above the
line
               allratio.append(p)

donnee('Results.csv')
donnee('Results1.csv')
donnee('Results2.csv')
donnee('Results3.csv')
donnee('Results4.csv')
donnee('Results5.csv')


fig = plt.figure()
plt.plot(Inten, allratio, 'g+', mew=3, ms=10)   # mew = marker edge width, ms = marker size
plt.xlim(-2, 102)
plt.ylim(0, 1.1)
plt.title("Development towards light or gravity in function of the intensity of light",
size=23)
plt.xlabel("Intensity of LED (%)", size=23)
plt.ylabel("ratio", size=23)
plt.show()
fig.savefig('Test1.png')
```

We will also **measure** the **maximal distance** of the fungus to the diameter line to calculate the speed of growth of our fungus. This maximal distance will be calculated for above and below the line. The used code will be:

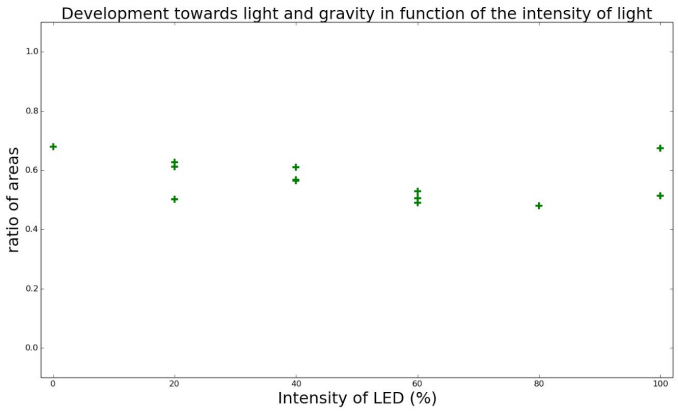
```
import numpy as np
import matplotlib.pyplot as plt
import matplotlib
from math import *
import csv

Inten = [0, 0, 0, 20, 20, 20, 40, 40, 40, 60, 60, 60, 80, 80, 80, 100, 100, 100]
allratio = []

def donnee(file):

    a = []
    data = file
    with open(data, 'rb') as csvfile: #Open the file
        reader = csv.reader(csvfile)
        reader.next() #switch the first line
        for row in reader:
               b=row[6]
               allratio.append(float(b))
    print(allratio)

donnee('Result1.csv')
donnee('Result2.csv')
donnee('Result3.csv')
donnee('Result4.csv')
donnee('Result5.csv')
donnee('Result6.csv')
print(allratio)


fig = plt.figure()
plt.plot(Inten, allratio, 'rx')
plt.xlim(-2, 102)
plt.title("Development towards light or gravity in function of the intensity of light")
plt.xlabel("Intensity of LED (%)")
plt.ylabel("maximum distance to line (mm)")
fig.savefig('Test2.png')
```

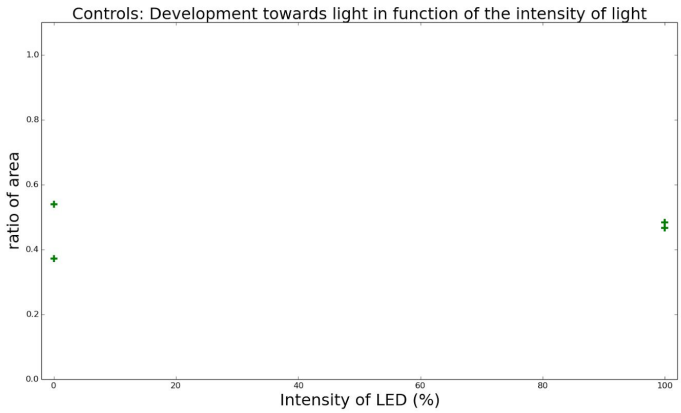
9/2/2017

Data gathering

This morning we gathered the data. We've made photos with a camera (Sony Reflex) and with a ruler next to i: to have a scale.

We've calculated the areas thanks to ImageJ: first the one below the line and then the one above. We've made a **.csv** for every intensity that then we parsed and plotted with our **.py**. Those were our results:



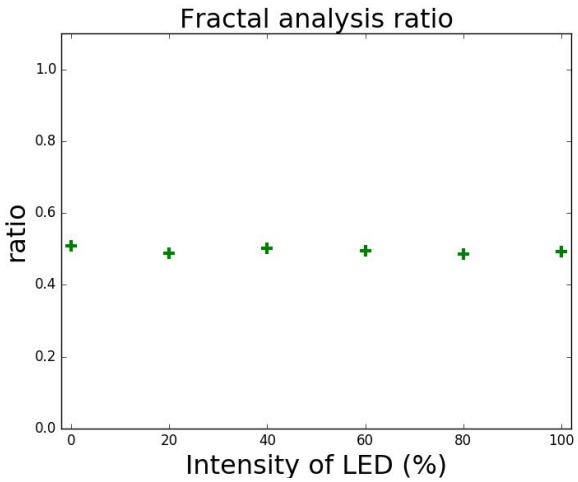


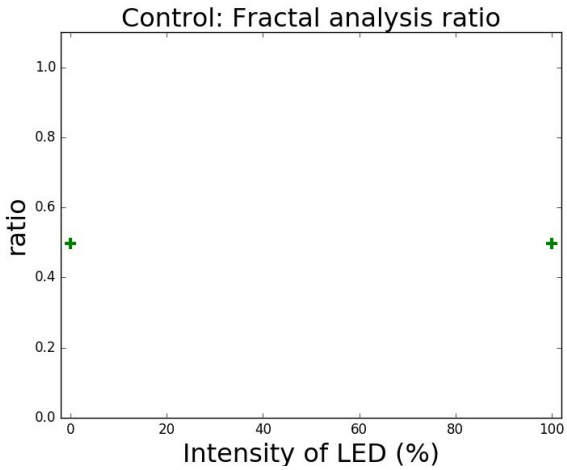
We can't see a clear tendency. Controls and replicates don't have a significant difference.

10/2/2017

Fractals

We've decided to use also fractals to analyze our data. To know the density of mycelium on each part of the plate. Those were our results:





We can't see a clear tendency. Controls and replicates don't have a significant difference.

Biais we had:

- Since we taped the plate with a really thick duck tape, we had to make holes in the plate with a needle to allow oxygen inside. They may not have enough oxygen to develop.
- The dark room can't stay in the dark all the time, therefore some light might go through the plate.
- The fungus takes 3 to 4 days to grow, therefore we might have to observe the results with the microscope.
- Maybe we did not take the same amount of colony during inoculation.
- Some of them were not inoculated properly, therefore, some of them may not have grown. These will be removed from data analysis.
- Sample size was not enough
- LED could have heat the plate: *Phycomyces* could have been in not their optimal temperatures.