Phototropism VS Geotropism in Phycomyces blakesleeanus

Final project report - Biosensors 2017
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Abstract

***Phycomyces blakesleeanus* is a fungus that does positive phototropism and negative gravitropism : it develops towards light but against gravity, thanks to its sporangiophores. In our project, we tried to find from which intensity of red light Phycomyces would mostly grow towards the light than against gravity. For that, we used opac plates and red LEDs, taped on a wall. After two days of experimentation, we collected and analysed the results on ImageJ. We measured the circumference of the organism and did a fractal analysis. Our results do not show any trends, probably because the experiment did not run long enough. We also had a lot of bias: while manipulating (inoculation) and while experimenting (light and hypoxia).**

Phycomyces blakesleeanus is a fungus from the zygomycota phylum. It is sensible to light, gravity and way more things like touch and wind. For example, when it senses a nearby object, *P. blakesleeanus* will change its growth direction as well as its growth speed in order to avoid it. *P. blakesleeanus* meiosis happen in sporangia, located in sporangiophores.

Sporangiophores are the part of the organism that senses light and gravity . We have decided to focus on this structure during our project; more precisely on phototropism and gravitropism. Phototropism can be defined as the ability of an organism to develop towards light while gravitropism as its ability to develop according to gravity. *P. blakesleeanus* does positive phototropism and negative gravitropism, meaning that it develops towards light but against gravity. Our main goal was to find a threshold from which positive phototropism would have more impact to Phycomyces than negative gravitropism.

Methods to test phototropism and gravitropism interaction

To test the interaction between phototropism and gravitropism we have attached Petri Dishes (inoculated of the organisms) to a wall. Then, put LEDs on the bottom part of those Plates. Gravity will make *P. blakesleeanus* grow up and light, grow down. We have tested different LED intensities to know the threshold from when phototropism is more important than gravitropism in Phycomyces. We have tested three conditions: 20%, 40% and 60% of total LED's intensity. We had two controls: at 0% of intensity (negative control) and 100% of intensity(positive control) (fig. 1.A). Gravity controls (that had also 0% and 100% of LED intensity) were placed horizontally in a table (fig. 1.B). We had done three replicates of each condition (for the six

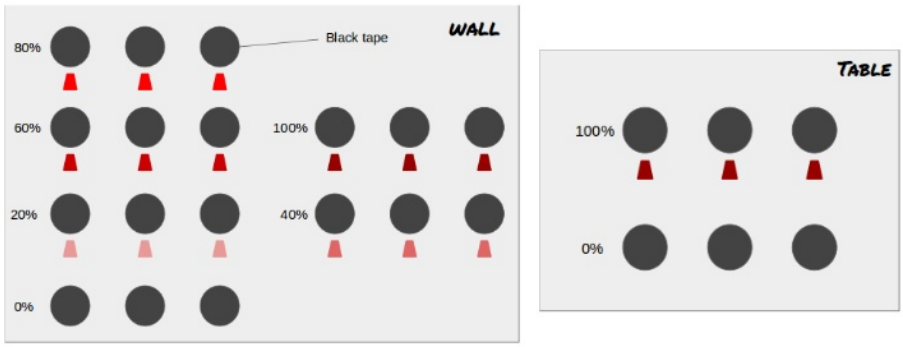

Fig 1.A & 1.B : ***Phycomyces plates setup.*** *A. How the setup is made on the wall, with the three replicates for each light intensity. B. gravity controls, put horizontally on the table.*

intensities on the wall and the two intensities on the table). To prepare those plates, we had done PDA (Potato Dextrose Agar) media (Supplementary Material p.10). To mark where organisms were inoculated and where the LED was situated we marked the diameter of the plate with a marker. This line was parallel to the floor. Then, we have done a little line from the center of the diameter that was directed towards the ceiling. We inoculated the organism where the little line and the diameter intersected (fig. 2).

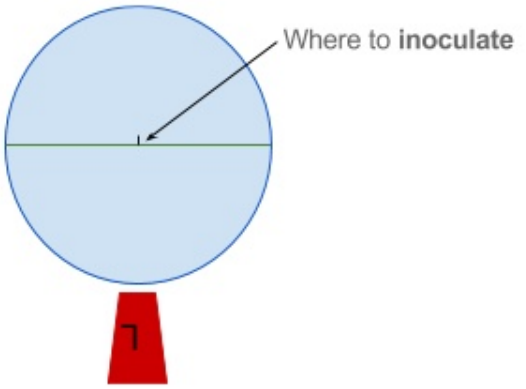

Fig 2: ***Plate setup.*** *We can see the diameter and the little line. The line is directed to the ceiling and the diameter parallel to the floor. The LED is on the bottom of the plate and* Phycomyces *is inoculated in the center of the plate.*

To inoculate Phycomyces, we rubbed the loop in the media and then in the center of the plate. We taped the plates with opaque tape and let a space on the bottom for the LED. We attached the plates to the wall with more tape, put the LEDs and put also opaque tape around the LEDs to block light from going anywhere but the plate they had to light up. Then we have pierced the plates in order to prevent hypoxia. We did an electric setup for each replicate (three LEDs) and then connected it all in different pins of the same arduino (fig. 3 and Supplementary material p.9) We let the experiment turn during two days and then gathered our data.

Analysing the organisms with two different methods : area analysis and fractal analysis

We decided to study the reaction of *P. blakesleeanus* in two different manners. One analysis of the area of development, and one analysis of the fractal dimension of the development (Supplementary Material p.8). For both methods, we used the image analysis software imageJ, allowing us to measure the area and the fractal number. The area analysis of the mycelium developed by *P. blakesleeanus* was the main idea of analysis, because it represented the easiest way to compare the impact of gravitropism and phototropism. We used the line that was drawn on the middle of every plate to compare the two areas of development. Since the organism was inoculated in the middle of the line, we planned to observe a clear distinction between the two areas in the direction of the light, since we found in literature that light induced a stronger response for growth than gravity. Therefore, we calculated ratios of the light attracted area (area under the line) on the gravity attracted area (area above the line). As shown in figures 4A and 4B, there doesn't seem to be any clear tendency in the development of *P.*

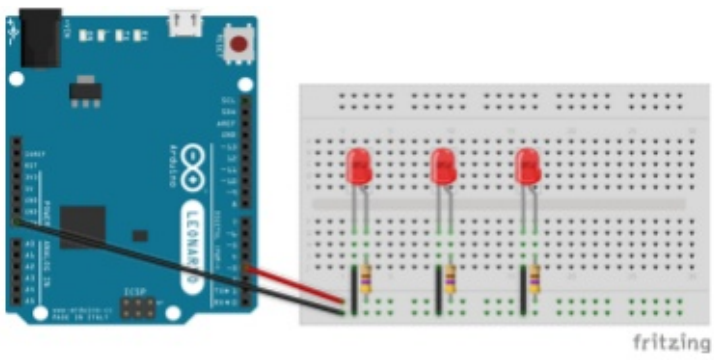

Fig 3: ***Arduino setup.*** *Arduino setup for three LEDs of the same intensity. This process was repeated for all the conditions.*

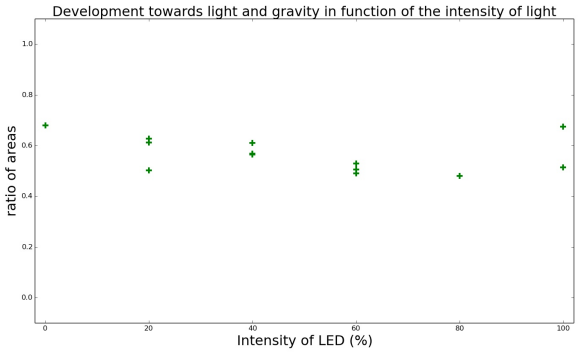

Fig 4.A : Development towards light and gravity in function of intensities of light. On the x axis, the different intensities of red LEDs and on the y axis, the ratio of areas measured. Each point, at each intensity, shows one of our replicates.

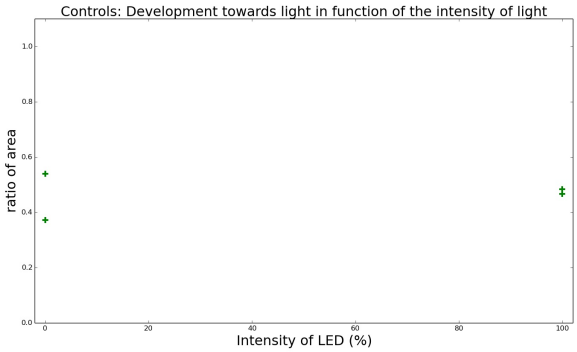

Fig 4.B : Development towards light and gravity in function of intensities of light - Controls. On the x axis, the different intensities of red LEDs and on the y axis, the ratio of areas measured. Each point, at each intensity, shows one of our replicates. This is our control graph, the plates that were put on the table.

blakesleeanus. Indeed, the intensities show no signs of great impact on the area development, as well as the horizontal controls which seem to have similar ratio as the vertical ones. This would indicate that neither light nor gravity had a significant impact on the area of growth of the fungus. No statistical analysis were done due to a lack of data and replicates.

Our second thought after observing no difference in area development was to study the density of development of *P. blakesleeanus*. In order to do this we measured the fractal dimension of the mycelium of the fungus. The method is more precisely explained in the Supplementary Material. Thanks to an imageJ plugin we were able to obtain the fractal number, a number between 1 and 2. with 1 describing a complex fractal dimension, which means a denser development. And 2 a simpler development, which means a hole-filled development.

As shown on figures 5A and 5B, there seems to be no tendency for this analysis as well. Indeed, the values for each intensities and for both horizontal and vertical controls seem to be the same, around 0.5. The fact that the values seem so close indicate again that there would be no impact of light and gravity on the density of development of *P. blakesleeanus*. Here again, no statistical test was done due to lack of data and replicates.

Discussions and conclusions

Our results may show that there seem to be no apparent link between light / gravity exposition and growth speed, numerous previous studies indicate the opposite. Therefore, we looked for bias and problems that occurred during the experiment. The most important one is that the sporangiophores of *P. blakesleeanus* take 5 to 7 days to grow, which means that light and gravity can only be sensed after this time. Moreover, we found during our experiment that gravitropism and phototropism would occur at different periods of development. Therefore our protocol would not be relevant to compare both sensed phenomena at the same time. Our experiment lasted for two days so sporangiophores didn't have the time to grow. Another factor that could cause a slow growing of Phycomyces was hypoxia. Since the only way the air had to enter in the plate was the holes we had made on the tape, maybe our organism grew in hypoxia conditions. Other limitations we had were the lack of replicates and the incertitude of having inoculate the same amount of organism on all the plates. We also were not completely sure that no light went into the plates apart the one from

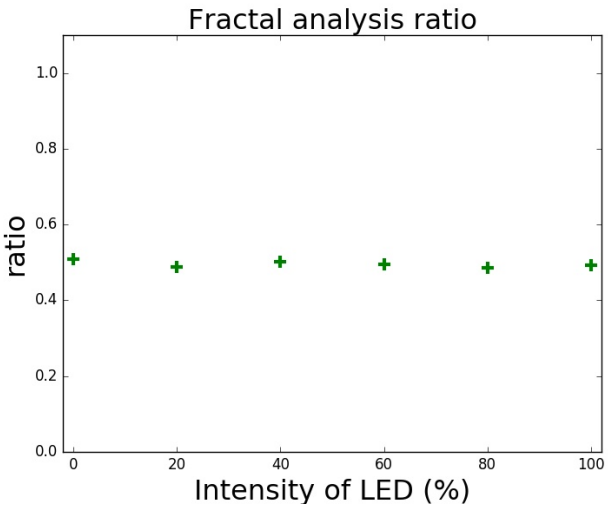

Fig 5.A : Development towards light and gravity in function of intensities of light. On the x axis, the different intensities of red LEDs and on the y axis, the ratio of fractal number measured. Each point, at each intensity, shows one of our replicates.

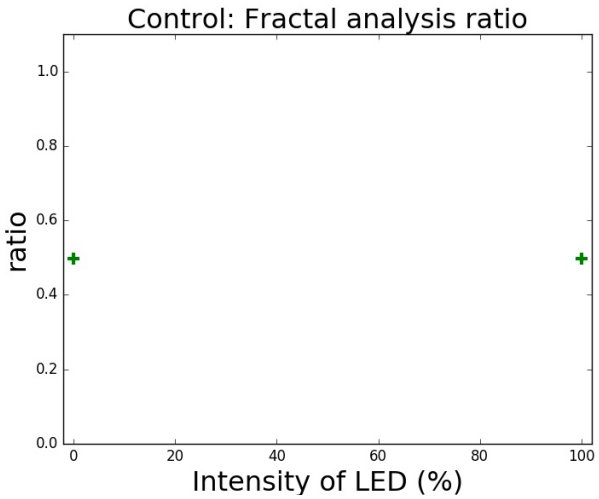

Fig 5.B : Development towards light and gravity in function of intensities of light - Controls. On the x axis, the different intensities of red LEDs and on the y axis, the ratio of fractal number measured. Each point, at each intensity, shows one of our replicates. This is our control graph, the plates that were put on the table.

the LEDs. We would have to do further experiments taking in account those variables to have more exploitable results.

This project is really important in our opinions since understanding the impact of factors such as light and gravity on growth means understanding how to control these factors. Indeed, knowing the thresholds and the limits of this impacts would allow the Research to optimize experiments, thus increasing the amount and the quality of work done on *P. blakesleeanus*. The comprehension of such vital parameters represents the comprehension of much more mechanisms of this fungus, and could even be extended to other similar fungi.

Special thanks to Tamara, Alice, Ivan, Lucy, Clément Caporal, Daniel and our mentors Eugenio Alcalde and Luis Corrochano. We would also like to thanks the CRI, the Frontiers of Life Science Bachelor and Foundation Bettencourt-Schueller to make possible this Biosensors 2017.

Sources

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Supplementary Material

Area analysis

Raw Data :

0 light intensity(LI) / 0 g (horizontal)
,Area,Mean,Min,Max
1,319.908,123.238,46,227
2,273.725,105.019,17,170
3,206.117,120.160,38,181
4,346.620,101.220,26,167

100 LI / 0g
,Area,Mean,Min,Max
1,375.374,90.124,21,169
2,401.724,100.230,14,169
3,333.054,104.144,20,220
4,381.522,107.636,12,200

0 LI
,Area,Mean,Min,Max,
1,23.867,117.472,32,161,
2,11.270,97.821,24,150,

20 LI
,Area,Mean,Min,Max,
3,301.481,84.358,11,212,
4,299.113,114.106,5,254,
5,144.349,89.007,2,230,
6,91.464,140.415,15,254,
7,404.135,111.343,11,254,
8,239.568,88.697,13,239,

40 LI
,Area,Mean,Min,Max
1,431.651,116.071,20,254
2,333.716,119.437,27,227

3,345.215,113.060,12,255
4,221.203,91.554,0,179
5,464.119,100.909,19,240
6,352.635,96.182,18,160

60 LI
,Area,Mean,Min,Max
1,442.331,111.514,12,248
2,459.614,111.773,13,199
3,419.951,108.925,24,188
4,412.040,110.181,5,166
5,553.848,121.704,13,253
6,493.920,143.663,27,255

80 LI
,Area,Mean,Min,Max
1,571.888,110.561,12,240
2,618.609,122.909,11,254

100 LI
,Area,Mean,Min,Max
1,383.979,105.355,14,170
2,364.349,104.556,12,152
3,549.527,111.960,32,245
4,266.192,141.350,15,225

Python code:

Area data

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

# Execute this program with Python2

Inten = [0, 20, 20, 20, 40, 40, 40, 60, 60, 60, 80, 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,len(a),2):         # Iterates on the pair numbers between 0 and 6:
0, 2, 4
              if a[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('0mur.csv')
donnee('20.csv')
donnee('40.csv')
donnee('60.csv')
donnee('80.csv')
donnee('100.csv')
print(allratio)

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, 1.1)
plt.title("Development towards light and gravity in function of the intensity of light",
size=23)
plt.xlabel("Intensity of LED (%)", size=23)
plt.ylabel("ratio of areas", size=23)
plt.show()
```

```
#fig.savefig('area.png')
```

Area control data

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

# Execute this program with Python2

Inten = [0, 0, 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,len(a),2):         # Iterates on the pair numbers between 0 and 6: 0,
2, 4
              if a[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('0l0g.csv')
donnee('100l0g.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("Controls: Development towards light in function of the intensity of light",
size=23)
plt.xlabel("Intensity of LED (%)", size=23)
plt.ylabel("ratio of area", size=23)
plt.show()
#fig.savefig('tablecontrols2.png')
```

Fractal analysis

Raw data :

1 = Under the middle line area
2 = Over the middle line area

0LI-1 : 1.7302
0LI-2 : 1.6720
0LI / 0g : 1.7936
0LI / 0g2 : 1.8122
20%-1 : 1.7570
20%-2 : 1.8352
40%-1 : 1.7808
40%-2 : 1.7607
60%-1 : 1.7969
60%-2 : 1.8397
80%-1 : 1.8012
80%-2 : 1.8985
100% / 0G1 : 1.7461
100% / 0G2 : 1.7714
100% / G2 : 1.7503
100% / G : 1.7037

Python codes

Fractal data

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

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

def donnee(file):
        fichier = open(file, 'rb')                  # opens the file
        a = fichier.readlines()
        c = []
        for row in a:
        b = row.strip().split()
        c.append(float(b[1]))
        for i in range(0,len(c),2):
        p = c[i]/(c[i+1]+c[i])  # make a pourcentage of the total area that is above the line
        allratio.append(p)

donnee('Log.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("Fractal analysis ratio", size=23)
plt.xlabel("Intensity of LED (%)", size=23)
plt.ylabel("ratio", size=23)
#plt.show()
fig.savefig('fractal.png')
```

Fractal control data

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

Inten = [0,100]
allratio = []

def donnee(file):
        fichier = open(file, 'rb')                  # opens the file
        a = fichier.readlines()
        c = []
        for row in a:
        b = row.strip().split()
        c.append(float(b[1]))
        for i in range(0,len(c),2):
        p = c[i]/(c[i+1]+c[i])  # make a pourcentage of the total area that is above the line
        allratio.append(p)

donnee('Logcontrol.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("Control: Fractal analysis ratio", size=23)
plt.xlabel("Intensity of LED (%)", size=23)
plt.ylabel("ratio", size=23)
#plt.show()
fig.savefig('fractalcontrol.png')
```

Fractal dimension analysis description

Quick definition

A fractal is a pattern that repeats itself creating an object called a fractal object. (example : A triangle composed of three triangles, also composed of three triangles, and so on.)
The fractal dimension of an object is the measure of the fractal patterns complexity that exists in it.
This complexity is defined by the capacity of the pattern to fill most of the place it takes, no matter at which scale.

This means that the analysis we performed described the density of development of the mycelium, since if the pattern is complex, its space-filling capacity increases.

ImageJ measures

In order to do a fractal analysis, we had to use an ImageJ plugin, called Fractal count.
To measure the fractal number thanks to this plugin, you must first focus on a part of your photo, the cleanest part that you can find in your photo, and apply different image modifications :
-Image : Type : 8 bit, in order to make the image in shades of grey
-Process : Binary : Make binary, to make the photo black and white (with the background in white)
if there is too much noise, try to : -Process : Binary : Skeletonize or Erode.
Then go to plugin and click on Fractalcount.
This plugin returns a number between 1 and 2,with 1 describing a complex fractal object and 2 a simple fractal object (rectangle).

With the number obtained, we calculate a ratio between the fractal number of the area under the middle line and the fractal number of the area above the middle line.

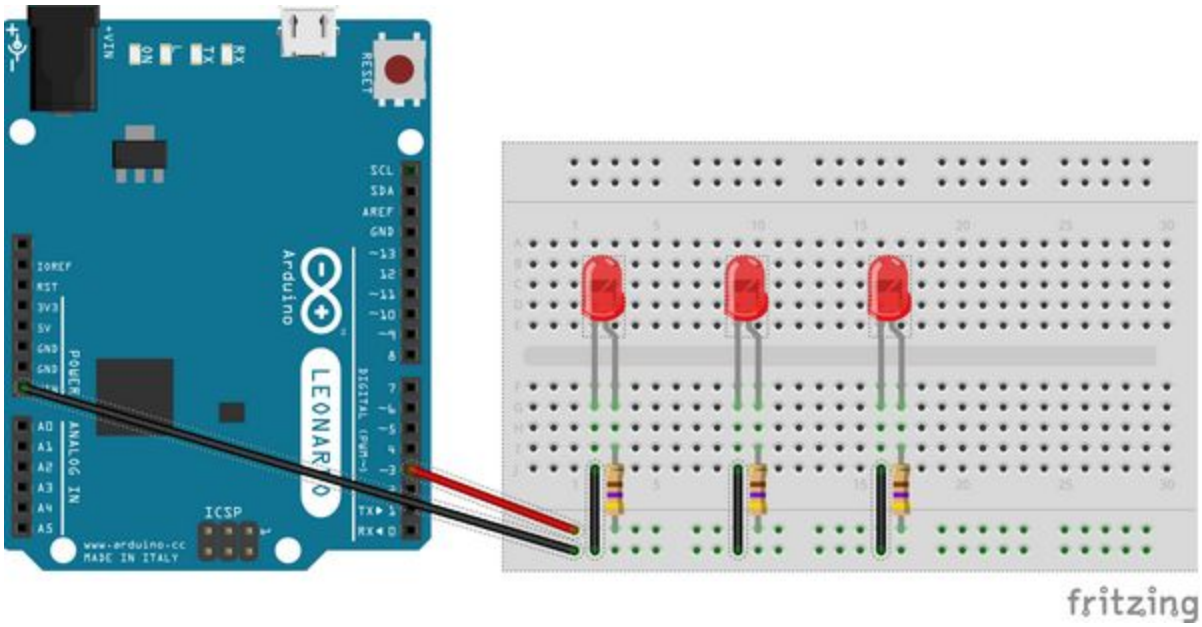
Arduino code and setup

```
//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);
}
```



PDA recipe

Material:

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

Method:

We boiled the sliced potatoes with the 1000 ml of osmotic water for 30 minutes. Once the potatoes boiled, we filter the liquid with a coffee filter. To be sure that the filtered liquid contained starch, we tested with 1 MxL^{-1} KI and the solution got blue. When the liquid is completely filtered, we added enough osmotic water to reach a liter of solution. Then, we added 20g of dextrose to the solution and then 20g of agar. We autoclaved it and poured it on 29 plates. We let it cool down 10 minutes and parafilm it to prevent it from being contaminated.