Effects of ginger on the growth of *Escherichia coli*

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Abstract

The aim of this scientific project was to determine the effects of ginger on the growth of non-pathogenic *Escherichia coli* bacteria. First the growth of the bacteria was observed on solid LB agar plate, where a 10% ginger-water solution was added to LB agar medium. The bacteria were diluted on control plates (without ginger) and secondly on the ginger plates. One could observe that ginger slightly influences the growth of the bacterial colonies in a negative way. Noteworthy was also the change of color from the bacterial colonies which grew under influence of ginger: The color of the colonies ranged from dark yellow to brown whereas the control bacteria were brighter.

A second research was done in a liquid culture where a high concentrated ginger solution was added. There, our results showed clearly that ginger, especially in high concentrations, inhibits the growth of *Escherichia coli*. Our hypothesis that ginger inhibits the growth of *Escherichia coli* is therefore confirmed

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1. Introduction

Our project wants to assess the ability of ginger to affect the growth of pathogenic bacteria (*Escherichia coli*).

Numerous drugs against colds and cough, as well as digestive problems often include ginger extract (for example the French medicine “Antimetil” by Thilman).
Therefore our idea was that ginger probably has an effect on the pathogens present in the human body. Naturally that wouldn’t have been possible to prove, so we decided to do our analyses on a model organism: a non-pathogenic strain of *Escherichia coli*.

2. Materials and Methods

Materials

Measuring Instruments

- Photometer
  Prim Light & Advanced Secomam
- Cuvettes
- Reflux condenser (to minimize water-loss)
  Phywe
- Soxhlet Extractor
  Phywe
- Rotary Evaporator
  Phywe
- 2 heatable magnetic stirrer
- 2 Temperature Gauge

Others

- Fresh ginger
- Strain culture Escherichia coli
  K12 Schlüter Biologie
- Ethanol
- Distillate Water
- Bunsen burner
- Oil bath
- Tripod
- Clamps
- Bossheads

Sterile Material

- Pipettes: 1ml, 2ml, 5ml and 10ml
- Micropipettes
- 4 Magnetic Stirrer
- 2 Round-Bottom Flask 250ml
- Drigalski spatula
- Inoculation Loop

LB-medium

- 5g/L Yeast Extract
- 5g/L Sodium chloride (NaCl)
- 10g/L Peptone

(+ 15g/L Agar- Agar for solid LB agar plates)

Methods

1) First culture

First of all, a culture of *E. coli* bacteria was established on LB-agar plates. That first culture of *E. coli* bacteria was obtained by using the strain culture.

With the help of an inoculation loop, the bacteria were taken of the tube and put in a round-bottom flask containing liquid LB-medium. 24 hours after their multiplying, 1ml was taken out with a sterile pipette and evenly spread out on an LB agar plate using a Drigalsky spatula.
This LB agar plate represented the primary culture.
For the following establishment of solid and liquid cultures, the bacteria were all taken out of that primary culture.

2) The cultures

Two types of cultures composed of a nutrient LB-medium, which enabled the reproduction of the *E. coli* bacteria, were used in this experiment.
To prepare the liquid LB-medium, one needs 5g/L of *Yeast Extract*, 5g/L *Sodium chloride (NaCl)*, 10g/L of *Peptone.* Those ingredients were diluted in 1L water and then sterilized by autoclavation.
To prepare the LB-medium for a solid culture, one only needs to add 15g/L of *Agar-Agar* to the preparation before the sterilization.

3) Solid culture

A first experiment was carried out to observe differences between *E. coli* bacteria growing on a normal solid LB-medium and a medium supplemented with a ginger aqueous extract.
During the preparation of one of the culture, a ginger solution was incorporated.
This aqueous solution was realized, by heating 10g of fresh ginger in 100ml distillated water. To minimize the loss of water, the ingredients were placed in a round-bottomed flask surmounted by condenser coil.
After the solidification of the LB agar plates, the bacteria were disposed on them via a dilution of $1*10^{0}$ to $1*10^{-10}$. Ten LB agar plates contained the ginger solution, while ten other plates without the ginger solution constituted a control.
This enabled to determine if an aqueous ginger solution, akin to a ginger tea extract was able to influence the growth of the *E. coli* bacteria.

4) Liquid culture – Growth of *E. coli* bacteria

The method chosen to analyze the growth of *E. coli* bacteria in presence of ginger was by measuring the absorbance of the liquid culture at 600 nm.

The measurement of the optical density can be related to the bacterial growth. To measure the growth of the bacteria, we measured the optical density of the culture every ten minutes. These measurements were used to establish a graphic analysis and determine the growth rate of the bacteria.

For the second part of this experiment a control growth and a growth in presence of ginger were carried out simultaneously.
The round-bottom flask containing LB-medium and a magnetic stir bar was heated to 37°C in an oil bath (set up on page 12). At the moment the fixed temperature was reached, the bacteria were incorporated to the LB-medium. When the latter reached their exponential growth phase (as determined by the first growth curve), the ginger solutions was added to the round-bottom flask.
The ginger concentrated extract used in this part of the experiment was made by extracting 6 g of fresh ginger in approximately 100ml ethanol (abs.). This extract was then concentrated using a rotational evaporator. After the maximal extraction of the ethanol, the remaining concentrate in the round-bottomed flask was diluted in approx. 2.5 ml of ethanol. (Ginger-ethanol extract)

It was apparent after this step that some additional fractions remained in the round-bottomed flask of the rotational evaporator. Approximately 2.5ml of water was used to dilute the remaining extract. Equivalent parts of both extracts were pooled and added to the liquid LB-medium.

A first set of experiments was carried out by using 1ml of the ginger-water-concentrate plus 1ml of the ginger-ethanol-concentrate incorporated into 200 ml of LB-medium.

A second set of experiments used a lower concentrated ginger (0.5ml of the ginger-water-solution plus 0.5ml ginger-ethanol-solution). This allowed assessing a difference in the growth of the bacteria between a high and a less concentrated ginger extract solution.

Here again, the growth of the bacteria was determined by measuring the optical density of the liquid culture.

After the measuring of both growths (one with and one without ginger extract), an additional test was realized to confirm the results obtained via optical density measurements. A dilution of both growth of $1*10^{0}$ to $1*10^{-5}$ was laid out on 5 LB agar plates.

3. Results

1) Solid culture

Figure 1: Bacteria grown on LB agar plates control conditions without ginger. Successive dilutions ($1*10^{-1}$-$1*10^{-10}$) from top left to bottom right

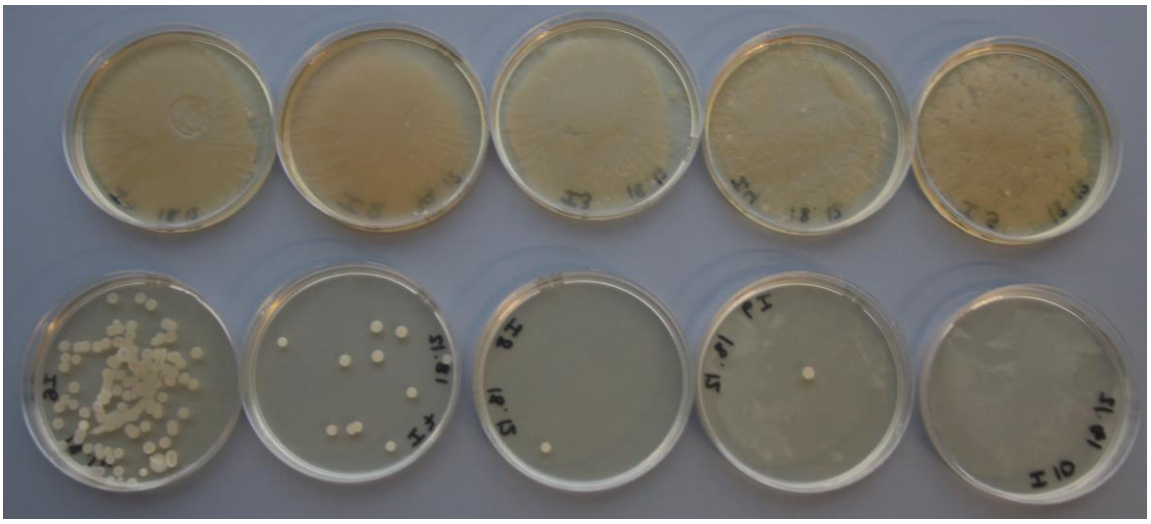

Figure 2: Bacteria grown on LB agar plates with aqueous ginger solution. Successive dilutions ($1*10^{-1}$-$1*10^{-10}$) from top left to bottom right. Note the smaller colonies and the different coloration of colonies

The observation shows a difference on the colour of the bacteria. The bacteria grown in presence of ginger are darker and slightly brown, whereas the control bacteria are more yellow and brighter.
Moreover it seems that colonies on ginger plates appear slightly smaller, indicating a growth reduction, as both plates were incubated during the same time.

2) Liquid culture

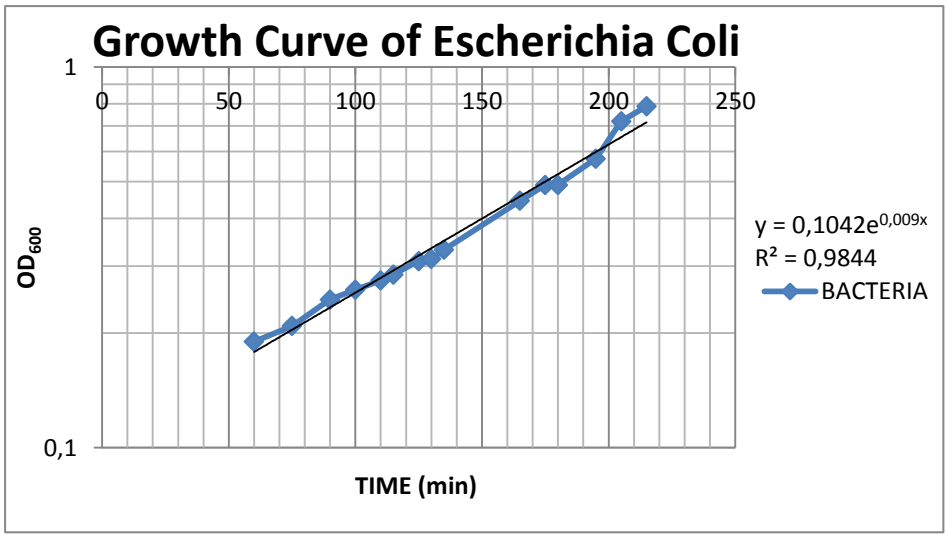

Figure3: Growth curve of E. coli in LB-medium.

During 3,5 hours, the growth of the *Escherichia coli* bacteria was determined by the measurement of the optical density (OD) at 600 nm. 60-270 minutes after the injection of the bacteria in the LB-medium, they reached their exponential phase. In all the following-up experiments, ginger-solutions were added in this exponential phase of time.

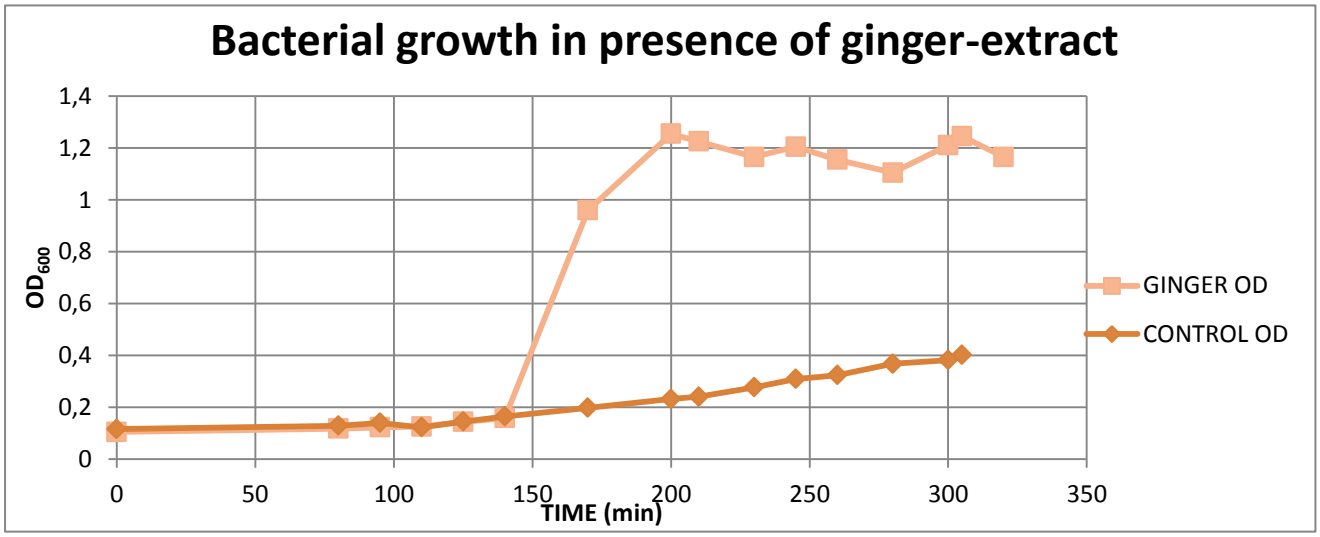

Figure 4: Ginger: Injection of 1ml ginger ethanol solution and 1ml water solution (after 3 hours of bacterial growth)
Control: Injection of 1ml ethanol (after 3 hours of bacterial growth)

At the addition of ginger one can note a significant change in the optical density of the medium (overall density changed from 0,2 to 1,2). This OD change can be attributed to the dark brown color of the ginger extract, which also visibly changed the color of the medium. (see pictures page 12)
After this initial change, attributed solely to the extract, the optical density of the medium with ginger remains stable which could indicate that ginger at this point inhibits the growth of *Escherichia coli* bacteria.

The control bacteria continued growing even with the addition of ethanol (added to account for the ethanol used in the ginger extract). Their optical density increased while the bacteria were in the exponential phase.

Proof:

Control bacteria without any influence of ginger:

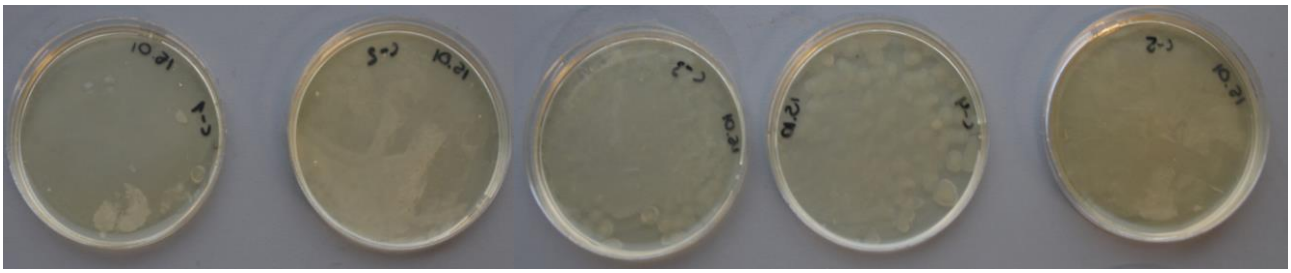

Figure 5: Control bacteria plates without any influence of ginger

Bacteria that grew under the influence of ginger:

Figure 6: Bacteria grown under influence of ginger

Comparison:

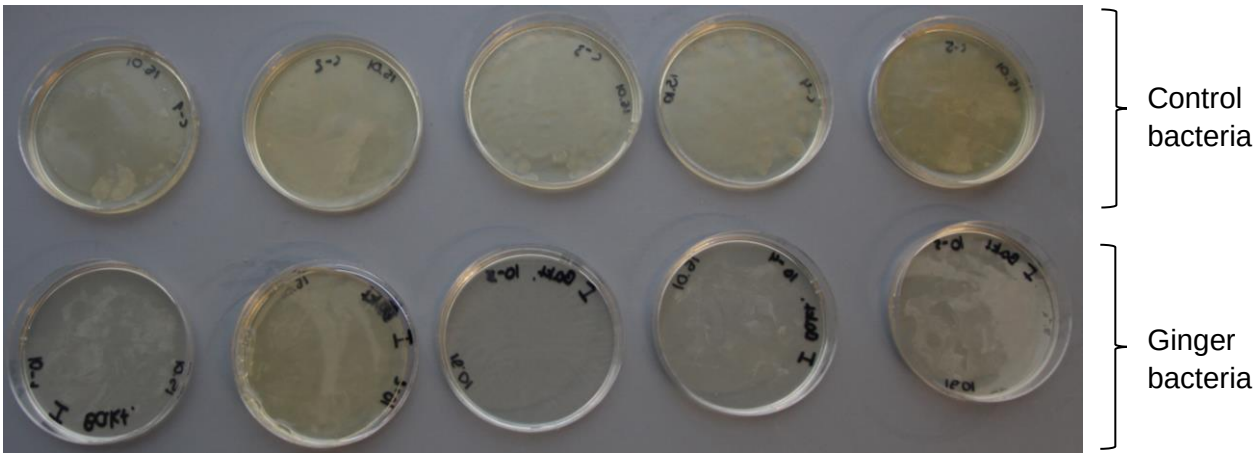

Figure 7: Control bacteria in comparison to ginger bacteria

One can observe that many bacterial cultures have grown on the control plates. Whereas no bacteria grew on the ginger plates*.[1]

[1] that brown traces on the agar plates with bacteria under influence of ginger are not a culture of *E. coli* bacteria but scratched up LB-medium with the inoculation loop

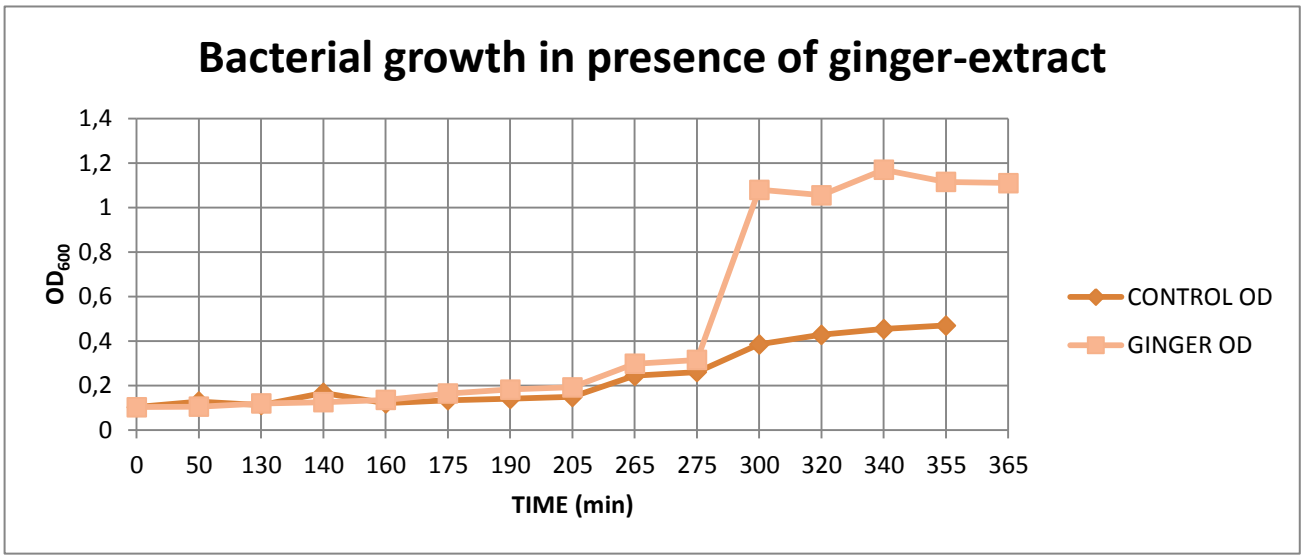

Figure 8: Ginger: Injection of 0,5ml ethanol solution and 0,5ml water solution (after 4,5 hours of bacterial growth)
Control: Injection of 0,5ml ethanol (after 4,5 hours of bacterial growth)

As before, at the addition of ginger one can note a significant change in the optical density of the medium (overall density changed from 0,2 to 1). This OD change can be attributed to the dark brown color of the ginger extract. After the addition of ginger to the bacteria their optical density didn't rise much anymore but stayed on a constant level. The control bacteria without ginger continued growing as their optical density continued increasing.

Proof

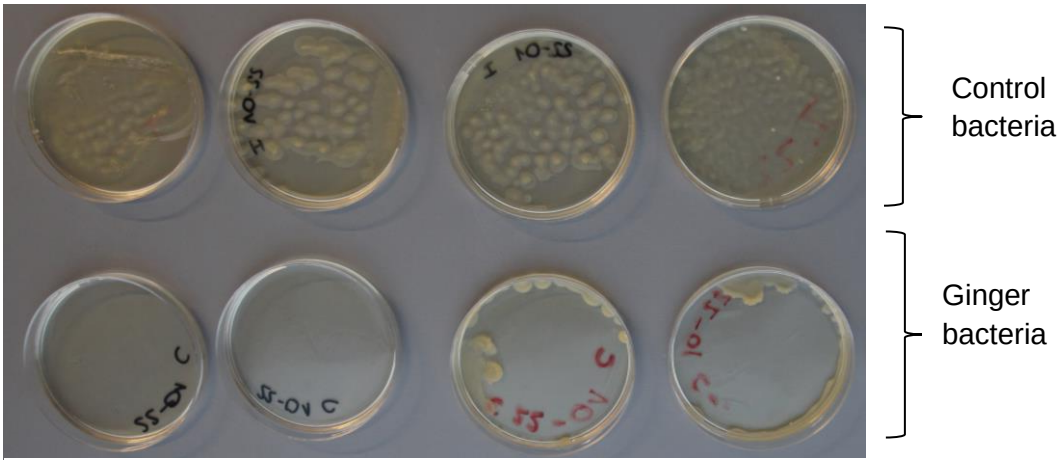

Figure 9 Successive dilutions of the control bacteria (top) and the ginger bacteria (bottom)

The bacteria that grew under the influence of ginger didn't duplicate on solid agar plates, therefore their growth is inhibit by the ginger. The control bacteria normally grew on the solid plates.[2]

[2] the traces on the agar plate are bacteria, the plates got contaminated (important amount of condensation water, medium too hot when poured), as can be seen that the bacteria grow near the edge of the plate

4. Discussion and Conclusion

Discussion

In a first experiment, *Escherichia coli* bacteria from the initial culture were placed in a liquid medium. After they had reached their exponential phase, 'a tea-like ginger-solution' (10g of ginger boiled in 100ml of distillate water) was added to the bacteria (data not shown). The growth of the bacteria didn't seem to be affected much in regard to the control bacteria without ginger. So, we came to conclude that the concentration of ginger was not high enough to see any effects on *E. coli*.
This experiment was repeated on LB agar plates (on 10 plates, 10ml of the 10% ginger solution was added instead of distillate water) and once more there were no clear effects visible.
Therefore, we tried to increase the quantity of ginger 'tea' in the solid agar plates, to see if that would disturb the growth of the bacteria. The LB-medium was now prepared with 50ml ginger extract and only 450ml distillate water (the control plates were prepared following the usual recipe). The bacteria were diluted (until 10^{-10}) and put on the agar plates. The results showed slight differences in colour and growth between the normally grown bacteria and those under influence of ginger. (see results on page 7)
For the next experiments, a self-made, high-concentrated ginger extract was prepared (see methods on page 6), and showed a clear inhibition of the bacteria.
These experiments showed us that ginger only has a visible effect on *Escherichia coli* in a high concentration.

Conclusion

1) Solid culture - External features

One effect of ginger on *E. coli* got visible on the solid bacteria cultures:
The bacteria which grew on agar plates with ginger had a darker, slightly yellow-orange color whereas the control bacteria were lighter, a slight impact on bacterial growth could be deduced from seemingly smaller colonies.
The change of color could be caused by an adaption of the metabolism of the bacteria to different potentially toxic substances contained in ginger. During this adaption phase bacterial growth would also be inhibited, which could explain the smaller colonies

2) Liquid culture – Growth of *E. coli* bacteria

The results of our experiences show that ginger has an antibacterial function against *Escherichia coli* bacteria. Especially in a high concentration, like the used ginger-extract in the liquid LB-medium, ginger inhibits the growth of *E. coli*.
This was primarily proved on the LB-agar plates containing the dilution of the bacteria under influence of ginger where no culture had grown, whereas the control bacteria normally grew.

5. Acknowledgments

At first we want to express our thanks to the FNR 'Fonds National de la Recherche Luxembourg' for their financial support. It helped us a lot in our research because we had the possibility to work with a new photometer which gave us more precise values so that we could improve our work and later on have more precise results.
Gratitude also goes to our school ALR 'Atert-Lycée Redange' and especially to the science department. Mr Benny Reuter prepared and offered us the material we needed for our experiments and overall we got support and help from all of the science teachers and also financial support from our school direction.
Thank you also to our tutor Mr Marc Olinger who supports us now for 3 years, where we participated to the National Young Scientist Contest in Luxembourg

6. Pictures

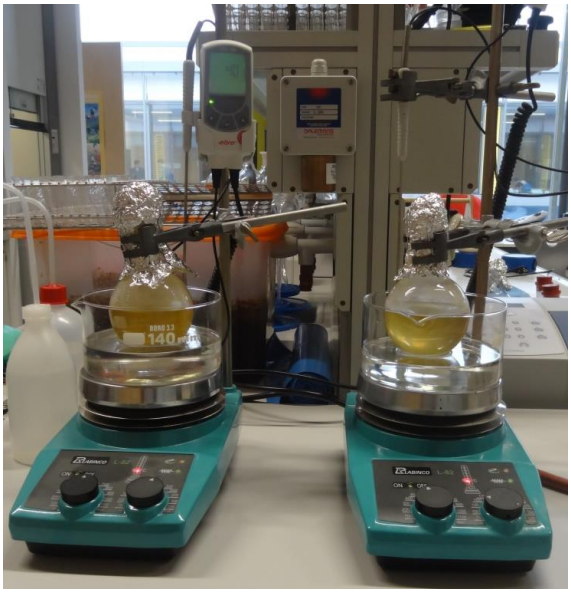

Figure 10 liquid culture set up

Figure 11 ginger extract

7. References

http://www.lifetechnologies.com/fr/fr/home/life-science/cell-culture/microbiological-culture/bacterial-growth-media/lb-broth-and-lb-agar.html