As part of my Senior Thesis Project, one of the aims or goals of my project was to be able to make my own nutrient agar petri dishes to complete my experiments in. I conducted my own research and designed my own method, collecting the suitable materials that would be the most beneficial for what I was trying to achieve.

Materials:

- Nutrient agar powder
- Plastic petri dishes
- Deionised water
- Nichrome loops
- UV steriliser
- Water deioniser
- Measuring cups
- Various beakers
- Weight measurement scales
- Sterilised wooden mixing spoon
- Kettle

By following the scientific method, I wanted to make sure that I could control as many variables as possible to create the most effective and accurate results in my thesis experiments.

Nutrient Agar Powder:

Each of the dishes used contains the same type of nutrient agar. Nutrient agar is found to be the most supportive of the growth of bacterial organisms. Nutrient agar contains several types of nutrients which aid the growth of bacteria. Peptone acid hydrolysate provides the necessary nitrogenous material for creating and growing bacterial cultures. Beef extract contains the necessary water-soluble substances which include carbohydrates, vitamins, and salts. The agar component of nutrient agar is a jelly-like substance which solidifies at room temperature, and creates a solid surface for bacteria to grow and create bacterial colonies.

Deionised Water:

All water used in the making of the petri dishes was deionised before being added to the agar powder as a method of standardisation. The minerals and ions that are commonly found in regular tap water, such as calcium and magnesium, may interfere with the nutrients found in the agar, or the actual growth of the bacteria when it comes to my thesis experiment. The water was treated to reduce and remove any pre-existing minerals and microorganisms to help ensure a consistent and pure medium.

UV Sterilisation:

All materials used, including the various beakers, measuring tools, plastic petri dishes, the agar powder itself, wooden mixing spoon, cups, and the nichrome loops were all sterilised using a UV (ultraviolet) steriliser for sterilisation. In a perfect world, all items would have been sterilised using an autoclave for the most accurate results of sterilisation, however, with

limited resources at my disposal, the UV steriliser is the next best thing. As a method of standardisation and to keep control of as many variables as possible, the sterilisation of equipment was an important factor which was kept at a high standard throughout the whole process.

All experiments were carried out wearing PPE (personal protective equipment) and clinical laboratory conditional standards were pursued to the best of my ability.

Petri Dish Experiment #1

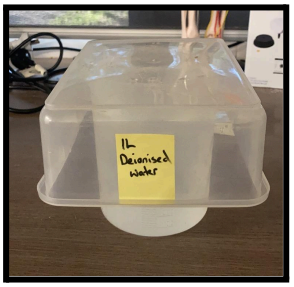
Method:

1. 1.5 litres of deionised water was made using a water deioniser.

2. All petri dishes, mixing and measuring equipment were sterilised using the UV steriliser.

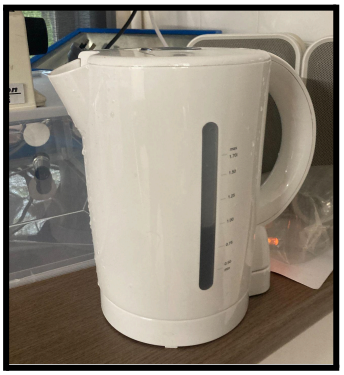
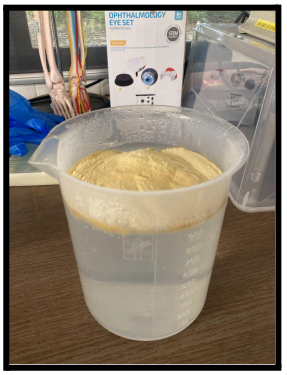
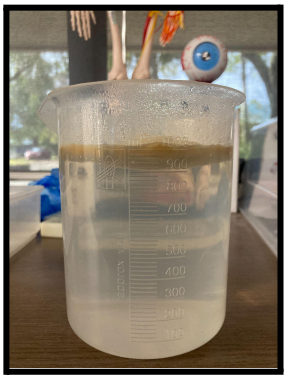
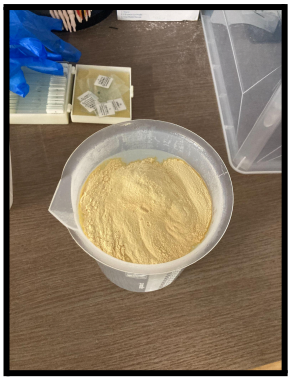
3. The deionised water was used to firstly rinse out the kettle and the beakers.

4. 1L of deionised water was then measured out into a beaker, and then boiled in the kettle for the maximum of 3 minutes, reaching a temperature of 100 degrees celsius (give or take 5 degrees).

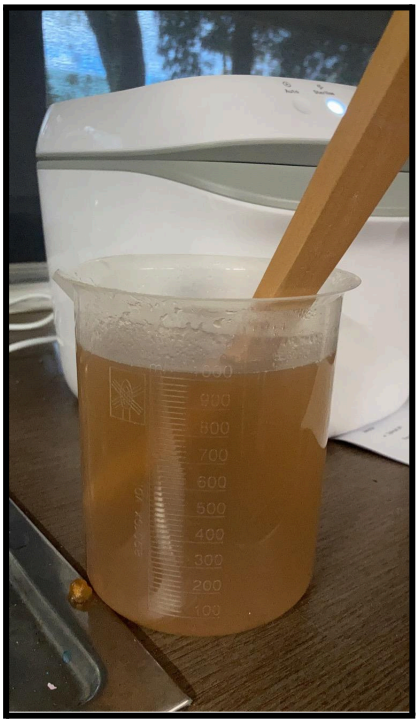


5. 29 grams of nutrient agar powder was weighed and measured into a spare petri dish.

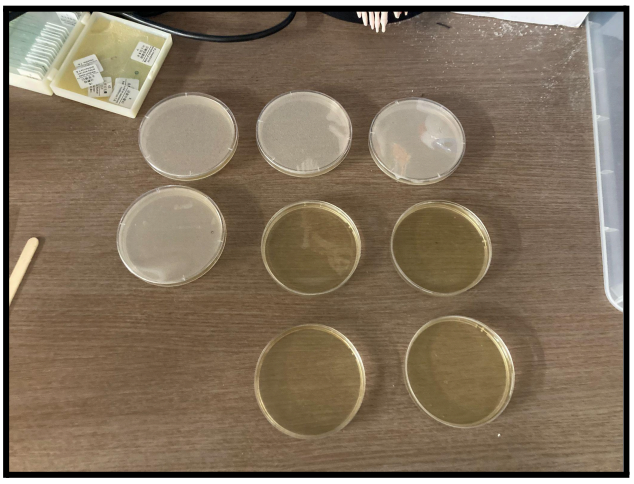
6. Once boiled, the deionised water was poured back into the beaker, and the measured agar powder was added to the beaker.

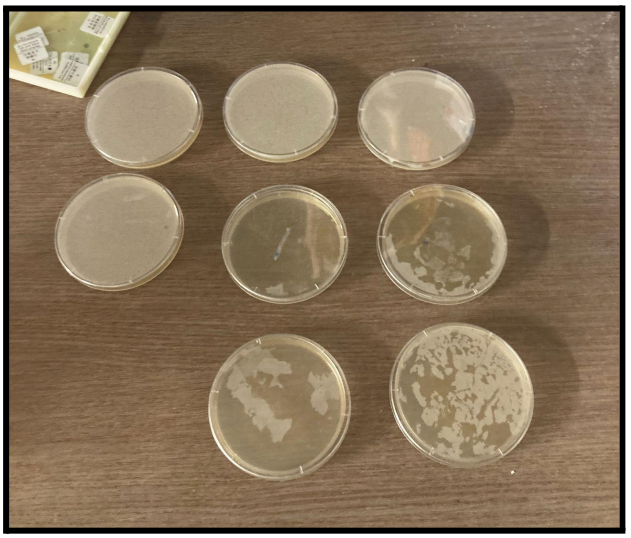
7. The materials were mixed together constantly until a thick gel-like consistency was met.




8. After all of the powder was dissolved in the water, each petri dish was then filled about halfway.

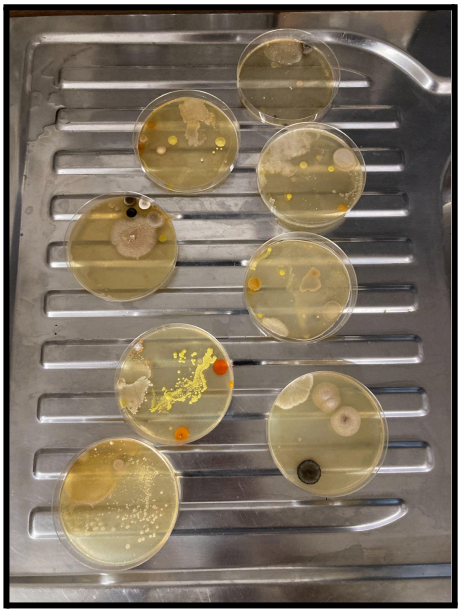


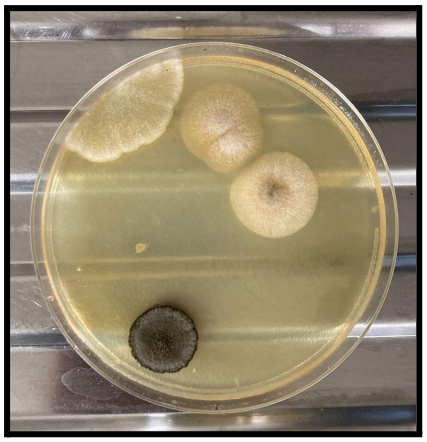
9. The lids of the dishes were put on and left to set.

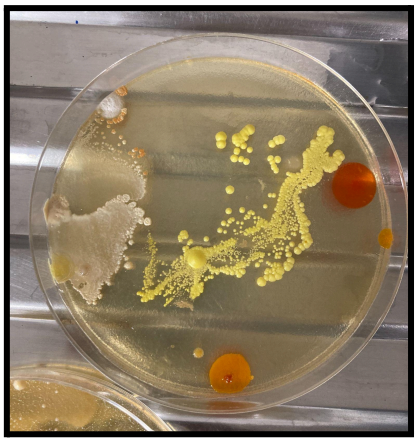


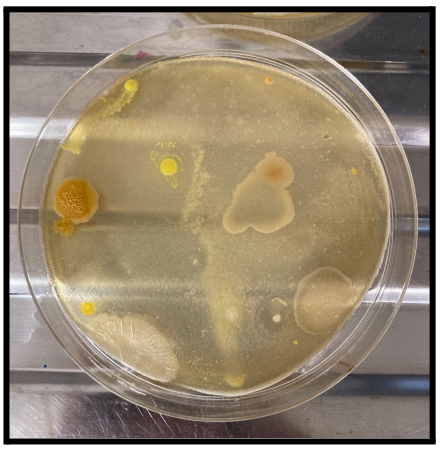
10. The dishes were then observed after 1 ½ weeks.

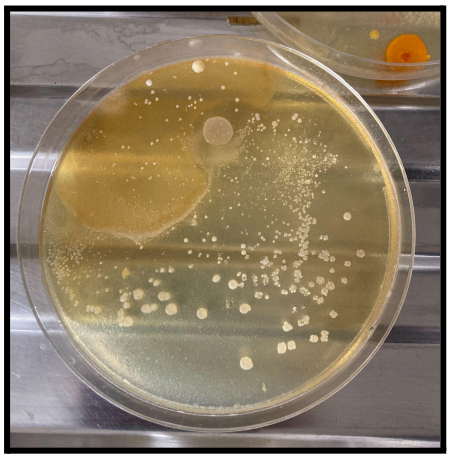
Results:
After setting, to test the effectiveness of the nutrient agar, half of the dishes were exposed to air, and the other half were left alone. None of the dishes were sealed completely. Results are as below.

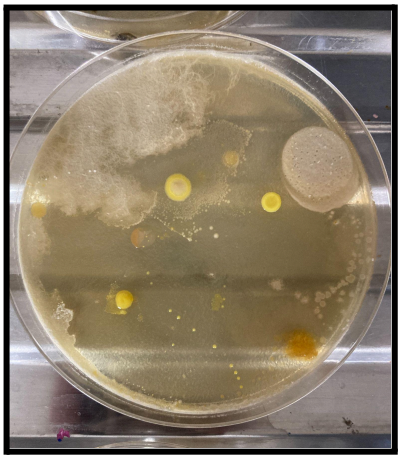


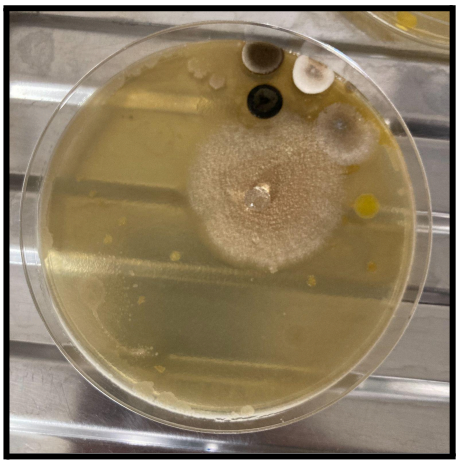


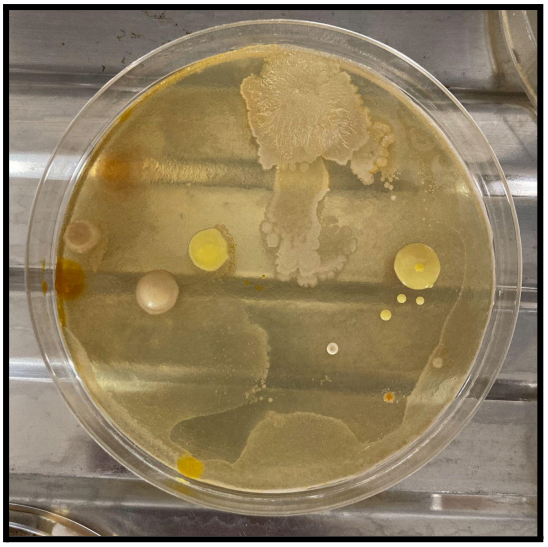


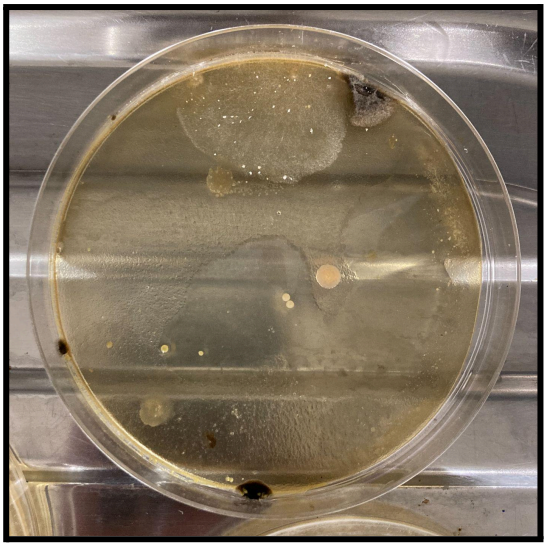












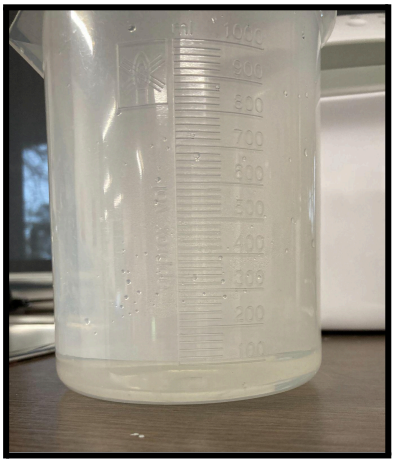
Each dish displays a diverse array of bacterial growth, ranging from different sizes, colours, textures and patterns of growth. In the end I found that this method for the petri dishes was quite successful.

Petri Dish Experiment #2

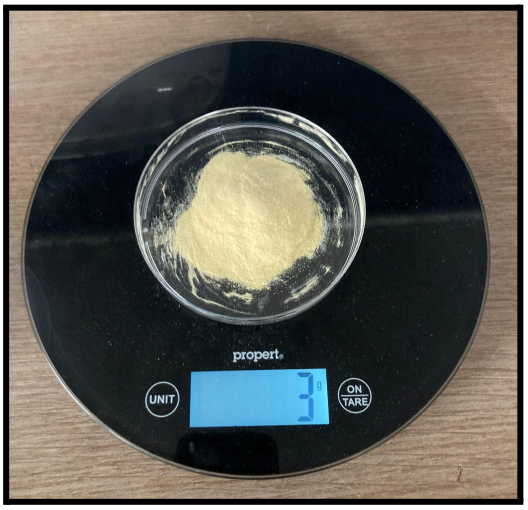
The aim of the second petri dish experiment was to be able to make a smaller batch of nutrient agar due to the amount that was leftover after the first experiment. The material measurements were divided accordingly to create ⅛ of the original batch.

Method:

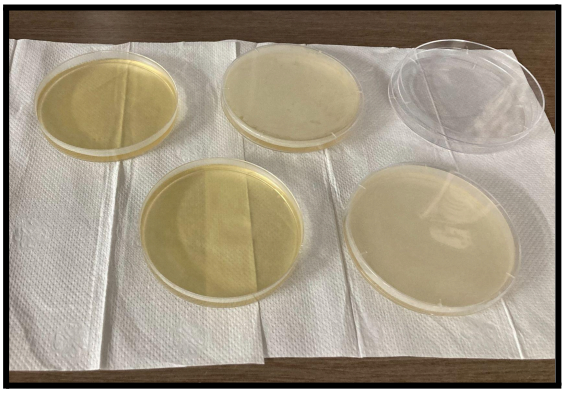
1. 150 millilitres of deionised water was made using a water deioniser.
2. All petri dishes, mixing and measuring equipment were sterilised using the UV steriliser.
3. 25 millilitres of the deionised water was used to firstly rinse out the kettle and the beakers.
4. 125ml of deionised water was then measured out into a beaker, and then boiled in the kettle for the maximum of 3 minutes, reaching a temperature of 100 degrees celsius (give or take 5 degrees).

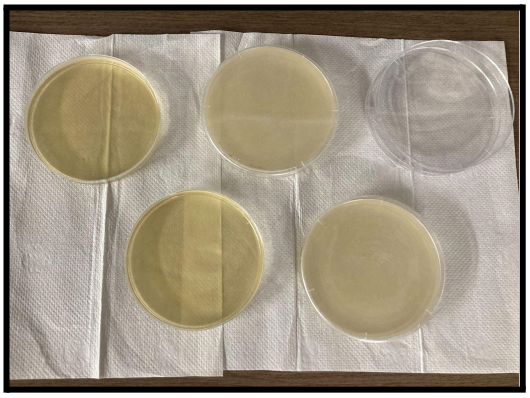


5. 3.5 grams of nutrient agar powder was weighed and measured into a spare petri dish.



6. Once boiled, the deionised water was poured back into the beaker, and the measured agar powder was added to the beaker.
7. The materials were mixed together constantly until a thick gel-like consistency was met.
8. After all of the powder was dissolved in the water, each petri dish was then filled about halfway.
9. Four dishes were made. To help determine whether or not it was best to put the lid on the dishes straight away after they had been poured, or to keep the lids off until the agar had set, two dishes were left open, and two dishes had the lids put on straight away.
10. All dishes were left sitting to cool down and set at room temperature and were observed after 1.5 hours. All lids were then put on and left for 15 hours.

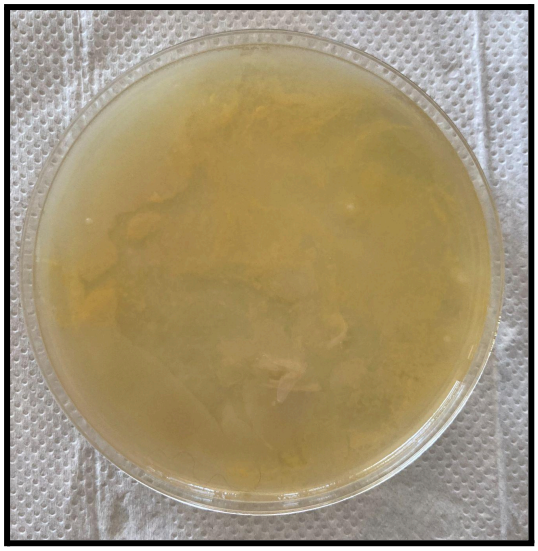




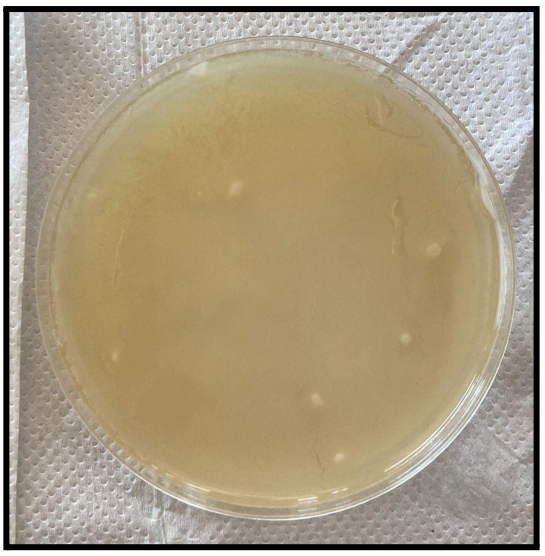
Results:

After the first observation, the agar was not set yet. All lids were placed onto the dishes and left for a further 15 hours. Upon observation 2, the agar was still not set. A thin layer on the top of the dishes had thickened, but not set completely.

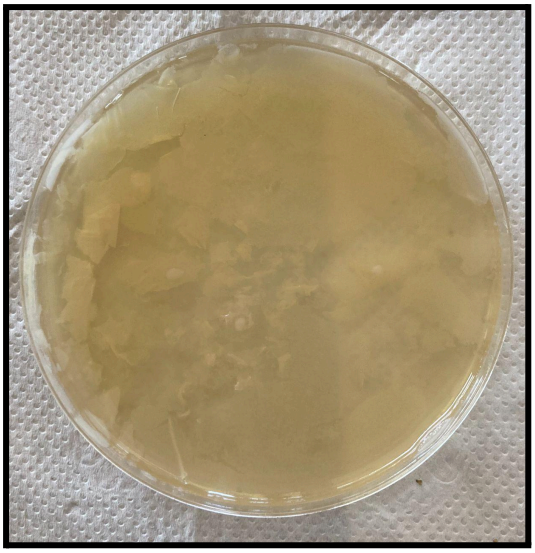
Due to this I was unable to determine whether the placement of the lid on the dishes straight after being poured, had a certain effect on the succession of bacterial growth on the agar.



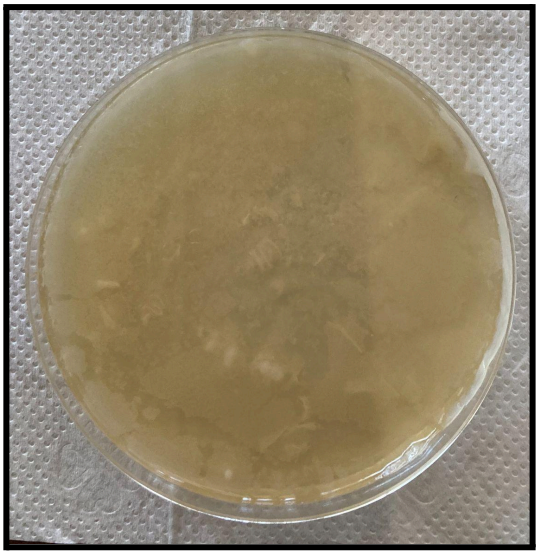
Dish #1 - No Lid Upon Pouring



Dish #2 - No Lid Upon Pouring



Dish #3 - Lid Upon Pouring



Dish #4 - Lid Upon Pouring

After the second batch of agar, I wanted to figure out why the agar was not setting correctly. I went over my calculations for the correct measurements in order to create ⅛ of the original batch, I had followed the exact same method I used for the original batch and went over all the variables and all seemed to have checked out.

Petri Dish Experiment #3

The aim of this experiment was to determine why the dishes were not setting after being brought down to a smaller portion size in the second batch.

After careful evaluation of the results from the prior experiment as well as the method of experimentation, the next step moving forward was to alter certain aspects of the method to help determine why the agar was not setting correctly.

Research was conducted, taking on different opinions and aspects, as well as comparing my own method from others who have made their own nutrient agar petri dishes in the past.

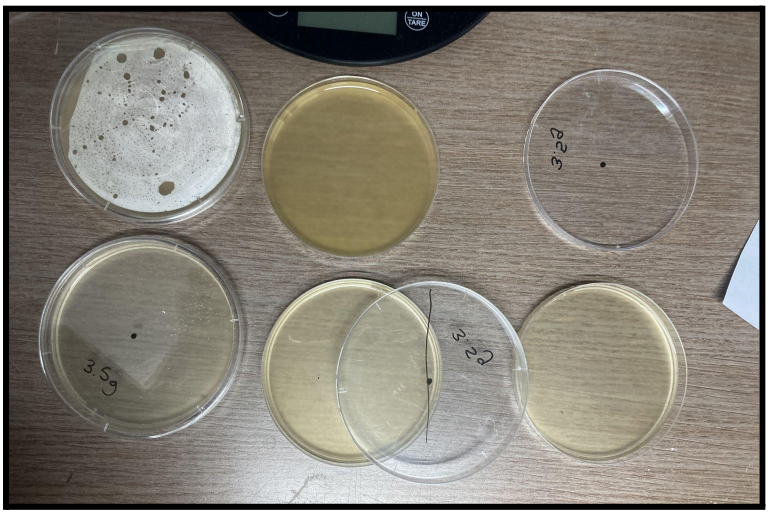
I found that most people had used an autoclave to sterilise the water-agar solution at 121 degrees celsius before pouring the dishes up, suggesting that heat plays a big role within this process. Due to the limited accessibility of resources for the project, I do not have access to an autoclave machine. As a substitute, I filled up the sink with boiling water, and continuously mixed the agar with the deionised water with the bottom of the beaker sitting in the boiling water.

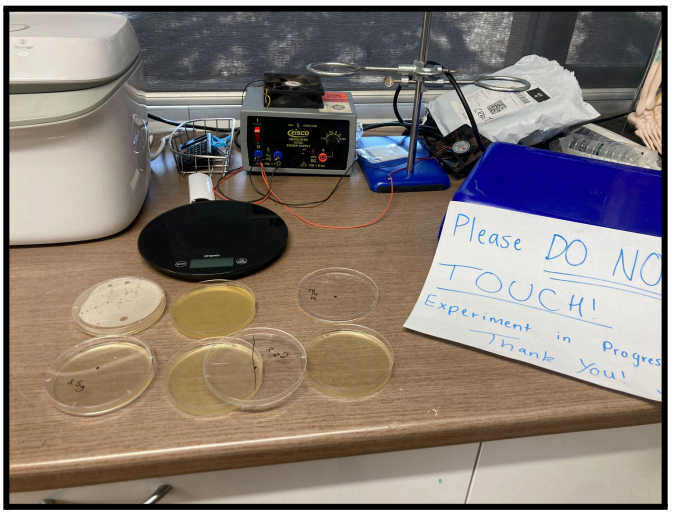
Other than this change, all steps from the previous experiment's methodology were kept at a constant.

Method:

1. 150 millilitres of deionised water was made using a water deioniser.
2. All petri dishes, mixing and measuring equipment were sterilised using the UV steriliser.
3. 25 millilitres of the deionised water was used to firstly rinse out the kettle and the beakers.
4. 125ml of deionised water was then measured out into a beaker, and then boiled in the kettle for the maximum of 3 minutes, reaching a temperature of 100 degrees celsius (give or take 5 degrees).
5. 3.5 grams of nutrient agar powder was weighed and measured into a spare petri dish.
6. Once boiled, the deionised water was poured back into the beaker, and the measured agar powder was added to the beaker.
7. The materials were mixed together constantly while sitting in the boiling water until a gel-like consistency started to form.
8. After all of the powder was dissolved in the water, each petri dish was then filled about halfway.
9. Five dishes were made. To help determine whether or not the agar was not setting due to factors within the method, 3 dishes were poured with 3.5 grams of agar being mixed into the deionised water and labelled.
10. To further experiment with the variables, 1 of the three 3.5gram dishes lid was put on straight away, 1 was left half on and half off, and the third dishes lid was left completely off.

11. After these dishes were poured, a further 0.5 grams of nutrient agar powder was added to the existing mixture, and was stirred constantly until the powder completely dissolved and the consistency of the mixture thickened.
12. The remaining two dishes were filled halfway. 1 dish's lid was put on straight away, the other remaining off.
13. All dishes were left sitting to cool down and set at room temperature and were observed 12 hours later.

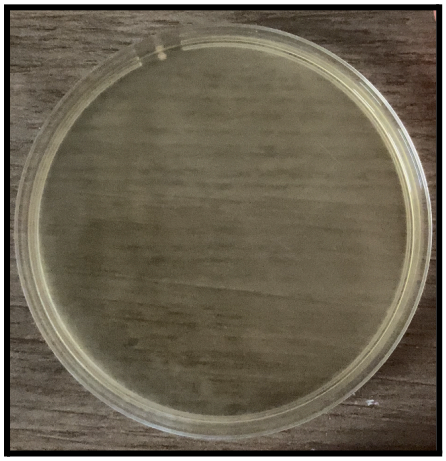




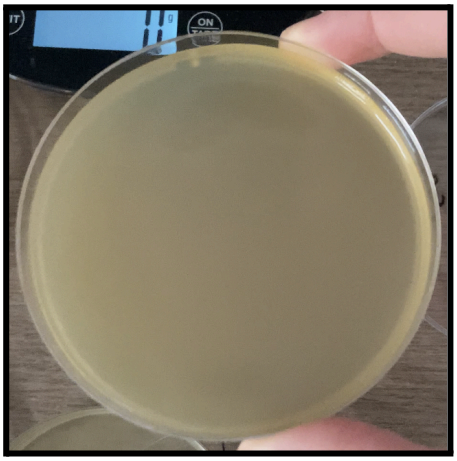
Poured dishes, ready to set.

Results:

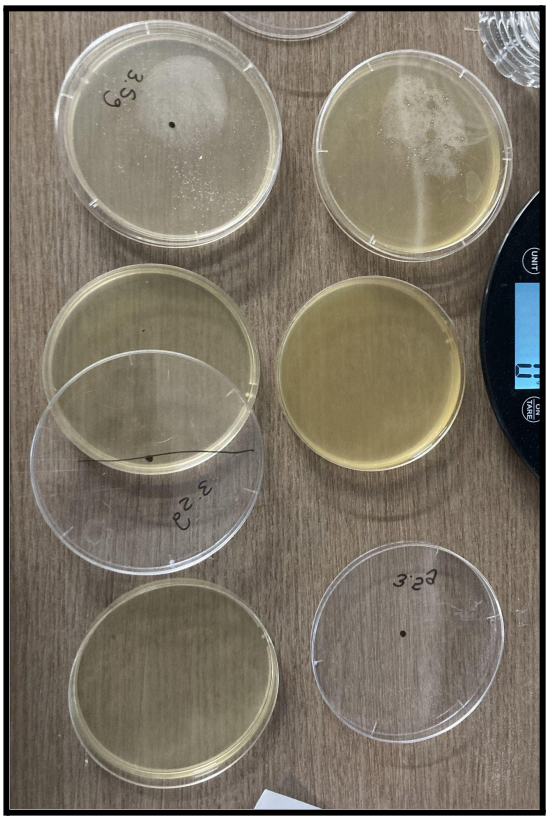
Upon observation 12 hours later, none of the dishes had set properly. The petri dishes made with a higher amount of agar powder (4g) were closer to setting and had a jelly-like thickness, compared to the dishes with 3.5 grams of agar powder.



3.5g of Agar



4g of agar



Overall Conclusion:

After a series of experiments, research, evaluations, observations, I am unable to conclude why the dishes from experiment #2 and #3 did not set. The methodology was carefully followed and kept as a constant until making considerate changes in the third experiment to help determine the cause of failure. The results from all three experiments overall, suggest that I may not have the most reliable materials to make my own nutrient agar petri dishes.

Moving forward, pre-made nutrient agar petri dishes were ordered and used for my thesis project investigation.