ARTIS MICROPIA

Probiotics

Full of beneficial bacteria

Many people have heard of Yakult, Vifit or Actimel. These are just a few examples of probiotic dairy drinks. 'Probiotic' refers to the fact that these drinks contain micro-organisms which are good for your health. One of the genera of bacteria commonly used in probiotic drinks is *Lactobacillus*, a lactic acid bacterium. The major difference between normal dairy drinks and the probiotic variety is that the bacteria in regular yoghurt are not able to survive in the stomach. The probiotic *Lactobacillus* bacteria are, however, able to withstand exposure to stomach acid and ultimately end up in the intestines, where they can support digestion and help create a healthy balance of intestinal flora.

According to the makers of Yakult, each bottle contains more than 20 billion bacteria. That is more than 300,000,000 per millilitre! In the following three experiments, we will try to culture these bacteria. The experiments can be conducted separately and have gradually increasing levels of difficulty. In the first experiment component, you will learn how to culture bacteria in a petri dish. In the second experiment, we will use a dilution series to see whether a single bottle of Yakult truly does have 20 billion bacteria. In the third experiment, we will look at what happens when certain fungi are exposed to these bacteria.

Part 1 (simple)

Culturing bacteria

You can culture bacteria in a petri dish with some growth medium, as a result of which bacterial colonies will become visible to the naked eye. This is referred to as inoculating bacteria. In this experiment, we will be looking at the bacteria in Yakult.

What do you need?

- a bottle of Yakult
- a pipette, 1ml
- a 10ml test tube with a stopper
- physiological saline solution (9 gram NaCl (table salt) in 1 litre of boiled water)
- a petri dish with growth medium (agar or gelatine)
- parafilm
- a sterile spatula or inoculation loop

If you are using agar as your growth medium, malt extract agar (MEA) is a good choice. It is also really fun to make your own gelatine-based growth medium. It is explained at the bottom of this test manual how to do that.

Getting started!

1. Fill the test tube with 10 ml of physiological saline solution.
2. Add a small drop (0.1 ml) of Yakult to this.
3. Place the stopper on the test tube and shake well.
4. Remove a small drop of liquid (0.1ml) from the test tube and streak this in the petri dish with the spatula.
5. Place the lid on the petri dish, seal it with parafilm and place it upside down in a warm, dry place, near the heater, for instance. For best results, you can use a drying cabinet or incubator for this. Set the temperature at 25° Celsius.

By quickly putting the lid back on the dish each time, you will prevent microbes from** the air ending up in your container. This is important, as they can 'contaminate' your **growth medium. Avoid exposure to airflows and draught. If necessary, you can also do the work in a fume cupboard or a box placed on its side.

6. After 5 to 7 days, have a look at the colonies which have grown in the dish.

Questions

Question 1: A whole bunch of colonies should have grown in the dish. From how many bacteria does a colony derive?

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Question 2: How many bacteria do you think there will be in the dish after seven days?

A just as many as the number of colonies

B thousands

C millions

D billions

Part 2 (average)

Counting billions

In this test component we will check whether a bottle of Yakult actually does contain at least 20 billion bacteria. You will do this by first diluting the Yakult before putting it in the petri dish. You can then calculate the total number of bacteria in a bottle based on the number of colonies in the dish.

What do you need?

- a bottle of Yakult
- a pipette, 1ml
- 5 10ml test tubes with stoppers
- physiological saline solution (9 gram NaCl (table salt) in 1 litre of boiled water)
- 2 petri dishes with growth medium (agar or gelatine)
- parafilm
- a sterile inoculation loop or spatula

Getting started!

1. Fill each test tube with 9 ml of physiological saline solution.
2. Pipette 1 ml of Yakult into the first test tube.
3. Mix the contents of the test tube by repeatedly sucking up the liquid with the pipette and releasing it again.
4. Place the stopper on the test tube and shake well. If desired, you can use a vortex.
5. Pipette 1 ml from the first test tube into the second test tube. Mix it again by sucking up the liquid with the pipette a few times and releasing it again. Shake well.
6. Repeat these steps until you reach the fifth test tube, by pipetting 1 ml from one test tube and adding it to the next one each time. Shake each test tube well before continuing to the next one. (see photo)
7. Remove 0.1 ml of liquid from the fifth test tube and use the inoculation loop to streak this in the petri dish. Make sure you put the lid back on the dish quickly, to prevent contamination.
8. Do this with the second petri dish too, so that you can compare the different results with each other.
9. Place the lids on the petri dishes, seal them with parafilm and place them upside down in a warm, dry place. You can also place them in a grow cabinet or incubator, with the temperature set at 25° Celsius.
10. Count how many colonies there are after 5 to 7 days.

Questions

Question 1: How many bacteria colonies do you count in the petri dishes? What is the average number?

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Question 2: If you add 1ml of Yakult to 9ml of saline solution, that 1ml of Yakult will be diluted ten-fold. By how much is the Yakult diluted in total?

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Question 3: Calculate how many bacteria there were in 1ml of Yakult. And approximately how many are there in a bottle?

TIP: Do not forget to take into account that you used 0.1ml in the petri dish, and not 1ml.

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Part 3 (advanced)

Fungus vs. bacteria

In this experiment, we are going to look at what happens when you inoculate bread mould and Yakult bacteria in the same dish. In step 1, we will inoculate fungi in a separate petri dish. In step 2, we will add this fungus to a petri dish containing Yakult bacteria. We are going to compare this dish with the petri dish to which amoxicillin has been added. Be careful not inhale too many spores when working with the fungus. It is a good idea to use a face mask.

Step 1: inoculating fungus

For this experiment, you will need a petri dish containing fungus. It takes about two weeks to prepare this. You can read here how to do that.

You will need the following to prepare this:

- a cotton swab with some household dust
- a slice of bread
- a plastic sandwich bag
- a petri dish with growth medium
- parafilm
- a pair of sterile tweezers

Getting started!

1. Sweep the cotton swab along an area with a lot of household dust.
2. Cut the slice of bread in half and sweep the cotton swab with the household dust over both sides.
3. Place the slice of bread in a plastic bag and tie the bag shut.
4. Let the bag sit for a week.
5. Look for a fungus spot which is white along the edges, but green-blue and fluffy in the middle. (see the photo) This is probably a *Penicillium* fungus.
6. Open the bag and use the tweezers to carefully remove a mouldy piece of bread.
7. Place this piece in the middle of the petri dish. Place the lid back on the dish and seal it with parafilm, so that no other microbes can contaminate it.

Let the petri dish sit for about a week in a warm, dry place. Then the fungus will be ready for use in step 2.

Step 2: fungus and bacteria in the same petri dish

What do you need?

- a bottle of Yakult
- a pipette, 1ml
- 4 10ml test tubes with stoppers
- physiological saline solution
- 2 petri dishes with growth medium (agar or homemade)
- a sterile inoculation loop or spatula
- a petri dish with *Penicillium* fungus
- a sterile spatula
- amoxicillin solution (a pinch of powder in 4ml of saline solution)

TIP: You can also use more petri dishes if, for instance, you would like to compare the effects of different types of fungi with each other.

Hypothesis

What do you think you will see?

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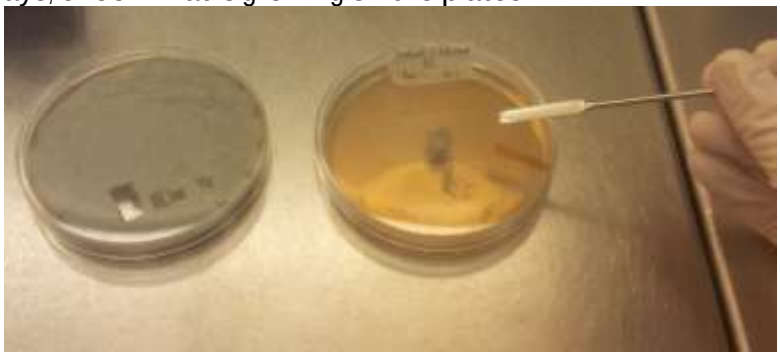
Getting started!

1. Dilute the Yakult in four test tubes as explained in steps 1 through 6 of experiment part 2.
2. Remove 0.1 ml of liquid from the fourth test tube and streak this in the petri dish. Make sure you put the lid back on quickly, to prevent contamination.
3. Use the sterile spatula to cut a small square of the growth medium with fungus on it out of the petri dish. Pick the piece up carefully and place it upside down on the first dish with bacteria (see the photo).
4. Also make a second plate with bacteria. Use the inoculation loop to carefully make a 1 cm slit in the middle of the plate halfway into the growth medium. Place a small drop (0.1ml) of the amoxicillin solution in the slit.
5. Place the lids on the petri dishes, seal them with parafilm and place them in the refrigerator with the growth medium facing down for 1 day (!). After that, place them in a warm, dry place with the growth medium facing up. You can also place them in a grow cabinet or incubator, with the temperature set at 25° Celsius.
6. After 5 to 7 days, check what is growing on the plates.



Questions

Question 1: What do you see happening to the fungus in the petri dish?

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Question 2: What do you see happening to the amoxicillin in the petri dish?

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Question 3: What could have caused this?

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Make your own growth medium (average level)

In order to carry out these experiments, you need a growth medium for the petri dishes. You can make this with agar, but it is also really fun to make a gelatine-based growth medium yourself. You can read how to do that below. With the quantities stated below, you will be able to make one litre of growth medium, which is enough to fill fifty petri dishes! It will take 5 days before the dishes will be ready for use.

You will need the following to prepare this:

- 50 petri dishes
- 60 grams of gelatine from 6 packages with 6 sheets each (total of 36 sheets of gelatine)
- 5 cubes of beef bouillon
- 5 tablespoons of sugar
- 1 litre of water
- a kettle
- a beaker or large bowl with a volume of at least 1 litre
- a refrigerator
- a beaker or bowl to soak the gelatine sheets in
- a tablespoon
- a mixing spoon
- kitchen paper
- aluminium foil

Getting started!

1. Boil 1 litre of water. In the meantime, continue on to the next steps.
2. Fill a bowl with cold water.
3. Separate the 36 gelatin sheets from each other. Place them together in the bowl with cold water.
4. Soak the gelatine sheets for 5 minutes in order to soften them.
5. Place 5 cubes of beef bouillon in the large beaker and add 5 tablespoons of sugar.
6. Pour the boiling water in the large bowl with the bouillon cubes and sugar.
7. Mix well.
8. Carefully wring out the soaked gelatine sheets so that there is almost no water left in them.
9. Place the gelatin sheets in the beaker with the mixture of water, bouillon and sugar.
10. Mix everything together until the gelatin has completely dissolved.
11. Carefully distribute the liquid over the petri dishes and place the lids on them as quickly as possible. This will prevent the liquid from being contaminated by the air.
12. Allow the mixture to cool to room temperature.
13. Once it has cooled off, place the dishes in the refrigerator for at least 4 days. While in the refrigerator, the gelatin will make the liquid turn into a solid jelly; the growth medium.

Four days later:

1. Remove the containers from the refrigerator.
2. Remove the lids. Wipe the droplets of water off of the inside of the lid with a piece of kitchen paper.
3. Then place the lid back on. Now you can use it for an experiment.