WJEC Wales Biology A Level

SP 3.4: Investigation into the numbers of bacteria in milk

Practical notes

Introduction

Fermented milks are dairy foods that have been **fermented** with **lactic acid bacteria**. They have not been pasteurised so **bacteria** remain and continue to **replicate** in them.

This experiment investigates the **number of bacteria** in fermented milk of **varying ages**.

Equipment

- Sample of fermented milk with a distant use-by date
- Sample of fermented milk at its use-by date
- 1 cm^3 syringe
- 9 cm^3 syringe
- 5× 9 cm Petri dishes
- Graduated pipette
- Molten agar cooled to 50°C
- 25°C incubator
- Distilled water
- 5× screw-cap bottles
- Sellotape
- Permanent marker
- Disinfectant

Risk assessment

Hazard	Risk	Precaution	Emergency
Pathogenic bacteria	Contamination of culture	Maintain aseptic techniques throughout; culture plates at 25°C	N/A
	Infection	Cover any cuts; wear disposable gloves and a lab coat; wash hands after practical; culture plates at 25°C (*not human body temperature*) do not re-open incubated plates; apparatus sterilised in a pressure cooker	Seek medical assistance
Disinfectant	Flammable	Make sure that there are no naked flames in the room	Put out small fires with a damp cloth; evacuate the building

Method

Sterilise all apparatus in a **pressure cooker** for 15 to 20 minutes prior to the experiment.
During the experiment, place all disposable items into a container labelled **'waste'**.
Once used, return all apparatus to the pressure cooker to be sterilised.

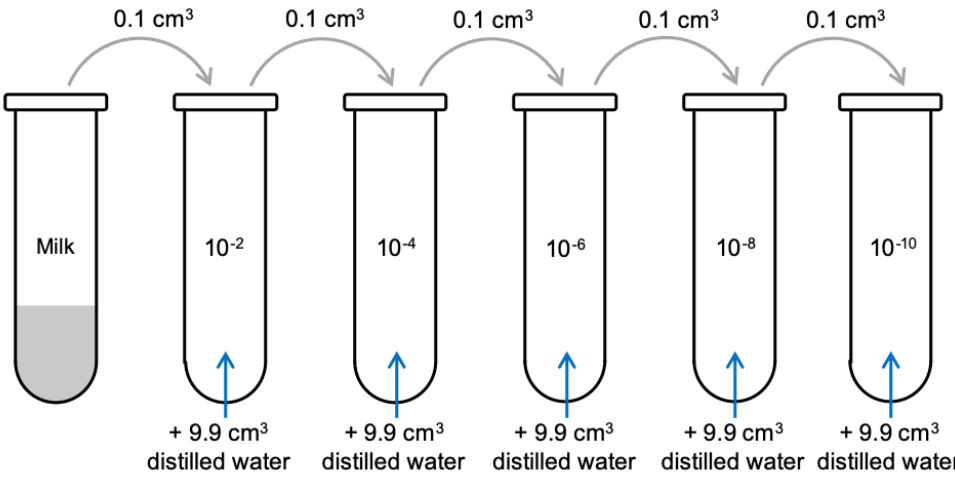
Carry out the following procedure for **both** samples of fermented milk:

Serial dilution

1. Start with the solution of fermented milk. Use a **graduated pipette** to transfer **0.1 cm^3** of the **milk** into a **screw-cap bottle** along with **9.9 cm^3** of **distilled water**. Label this **10^{-2}**.

2. Next, use a graduated pipette to transfer **0.1 cm^3** of the **10^{-2}** solution into a screw-cap bottle along with **9.9 cm^3** of **distilled water**. Label this **10^{-4}**.

3. Repeat until a **10^{-10}** solution is produced:



4. **Swirl** each screw-cap bottle to gently mix

Culturing bacteria

1. Take five sterile Petri dishes and label with 10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} and 10^{-10}.

2. Using a graduated pipette, add **1 cm^3** of the **10^{-2} milk dilution** and **12 cm^3** of **molten MRS agar** into the centre of the corresponding Petri dish. Swirl to mix and to evenly cover the bottom of the dish. *Ensure that the contents of the beaker are not transferred to the sides of the dish.*

3. Add 2 to 4 pieces of **sticky tape** to tape the base of the Petri dish to the lid.

4. Repeat steps 2 to 4 for the four other milk dilutions.

5. Leave the Petri dishes at room temperature until the agar has solidified.

6. Incubate at **25°C** for **five days**. A temperature of *25°C discourages the growth of bacteria pathogenic to humans. Store upside down to prevent condensation disrupting the culture.*

Bacterial count

1. Remove the Petri dishes from the incubator. Observe the plates and select the dilution that produces the most **distinct** colonies.

2. Count the number of **bacterial colonies** present on the selected plate. *Use a marker to highlight each colony counted on the Petri dish to prevent re-counting.*

3. **Estimate** the **bacterial count** of the **initial** fermented milk sample. *Each bacterial colony arises from a single cell, enabling the estimation of the number of cells in the initial culture.*

   e.g. 56 colonies counted on the 10^{-6} dilution Petri dish
   $\therefore 56 \times 10^6 = 5.6 \times 10^7$ bacteria per cm^3 fermented milk.

To increase the reliability of the results, the experiment can be repeated a further two times for the dilution that produced the most **distinct** colonies. This gives three bacterial colony counts, enabling the calculation of a **mean**.

Conclusion

The expected result is that **milk at its use-by date** will have **greater numbers of bacteria per cm^3** than milk with a distant use-by date. The increased length of time from production allows a greater period of time for reproduction.