

Yoghurt with another difference

DAVID MISKIN, UNIVERSITY OF HUMBERSIDE

MILK IS ROUTINELY tested for residual antibiotics — not because these pose any health risk, but because their presence may prevent the growth of starter culture organisms used in, for example, the manufacture of cheese or yoghurt. The way in which Penicillin G interferes with cell division can easily be demonstrated in the school laboratory, using Lactobacilli and Streptococci from yoghurt. This work provides a stimulating context for carrying out some basic microbiological techniques: Gram's staining and direct cell counts.

Materials

Penicillin G discs
(from the usual school science suppliers)
UHT milk
Non-Pasteurized plain natural yoghurt suitable for use as a starter culture (different supermarket brands seem to vary considerably, so trials might be needed to determine the best type to use)
Microscope slide
'Chinagraph' grease pencil
Crystal violet solution, *made up as follows:*
Add 2 g of crystal violet to 100 cm³ absolute alcohol. Make up a second solution of 1 g of ammonium oxalate in 100 cm³ of distilled water. Add 25 cm³ of the first solution to 100 cm³ of the second.
Iodine solution, *made up with 1 g of iodine and 2 g of potassium iodide in 300 cm³ of distilled water.*
Ethanol (95%) — laboratory IMS will do
1% safranin solution, aqueous
Microscope with x100 (oil immersion) objective
Stage micrometer
Incubator or water bath at 40°C
Home-made micropipette to dispense 0.01 cm³ liquid (see NCBE *Newsletter* 10 for construction details)

Practical details

Making the yoghurt

1. Warm the UHT milk to about 40°C (±2°C).
2. Add Penicillin G discs to the milk (up to 8 per 500 cm³ of milk).
3. Stir in 2% v/v starter culture.
4. Incubate for 4–6 hours without stirring at 40°C (±2°C).

Preparing a heat-fixed slide of bacteria from the yoghurt

1. Pass a dry slide through a Bunsen burner flame to remove any grease.
2. Place the slide over a sheet of graph paper marked in square centimetres. With a well-sharpened grease ('Chinagraph') pencil mark out on the slide two squares of 1 cm² each.
3. Mix the suspension of yoghurt thoroughly and use the micropipette to transfer exactly 0.01 cm³ of suspension into the centre of each marked area.
4. Using a sterile straight wire spread the suspension evenly over the marked areas.
5. Keeping the slide horizontal, dry the film rapidly

in the air near the flame, then heat fix the bacteria onto the slide by heating it momentarily in the Bunsen flame.

Staining the bacteria on the slide (Gram's stain)

1. Cover the heat-fixed film on the slide with ammonium oxalate crystal violet solution and stain for 30 seconds.
2. Rinse off the stain with tap water.
3. Wash off the water using iodine solution. Cover the film with the iodine solution for 30 seconds.
4. Rinse off the iodine solution with tap water.
5. Wash away the water with ethanol and decolorize the slide until the washings are pale violet – do not over-decolorize.
6. Wash away the ethanol with tap water. Gently blot or air dry the slide.

Examining and counting the bacteria on the slide

1. Set up the microscope and use the x 100 oil immersion objective. Use the stage micrometer to determine the field of view, and hence the number of fields in 1 cm².
2. Examine the slide under an oil immersion lens and count individual organisms, pairs chains and clumps. Any organisms which are aggregated should be counted as one clump; organisms that are more than the length of one bacterium away from the clump should be counted as individuals. The number of fields to be counted depend on the number of organisms present in each field:

Average number of clumps or organisms per field	Number of fields to be counted
0–3	64
4–6	32
7–12	16
13–25	8
26–50	4
51–100	2
>100	1

3. Count the organisms present in both marked areas on the slide and determine the average number of organisms and clumps per field. Let the average count per field = N. Let the number of fields in 1 cm² = A.

No. of organisms in 1 cm² (i.e. 0.01 cm³) = N × A
 No. of organisms per cm³ of yoghurt = N × A × 100
 Should the suspension be too dense to count directly, dilute it as necessary (1:10 or 1:100).

Safety

The yoghurt produced in this way *should not be tasted*. Caution should be exercised when handling ethanol (keep away from flames!) or the stains e.g. crystal violet.

Further activities

1. Yoghurt bacteria can be also be stained using methylene blue solution. The dye should be left on the slide for 2 minutes, then gently rinsed off with water. The slide should be air dried (without blotting) before examination.

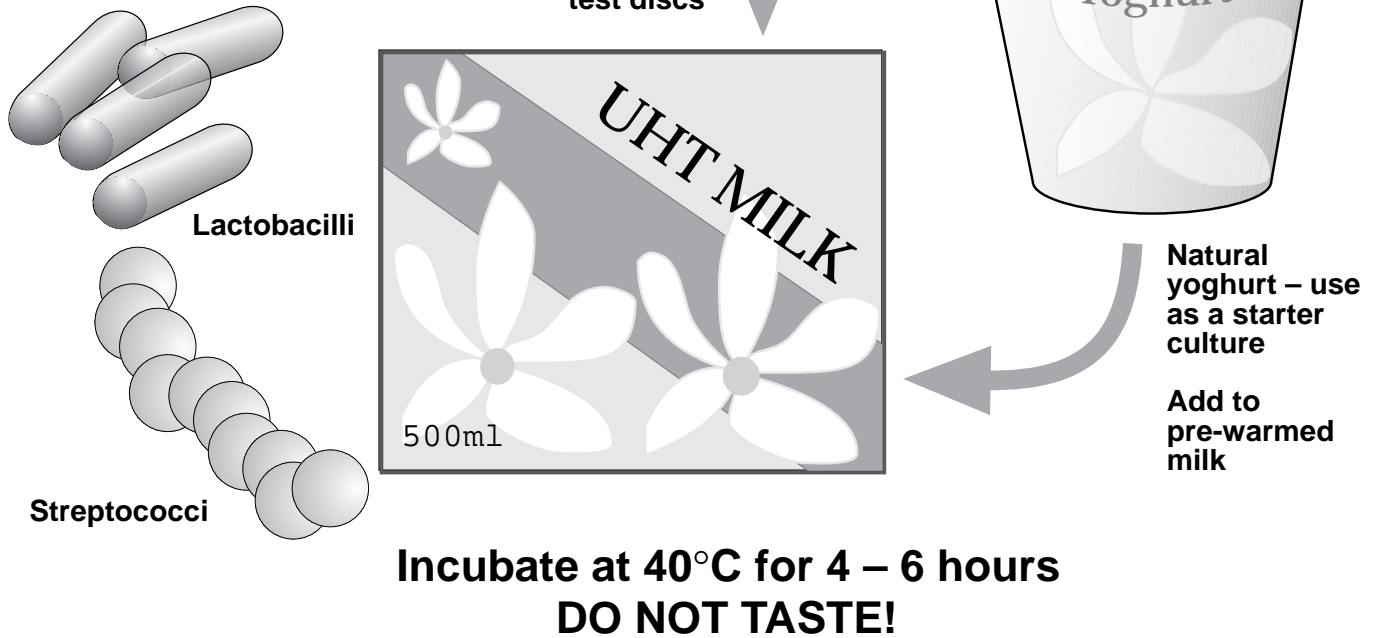
ADDITIONAL INFORMATION

The NCBE is grateful to David Miskin for giving us this original idea.

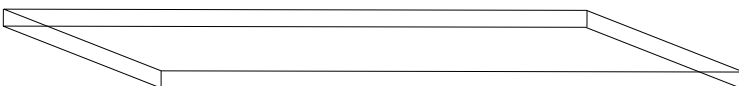

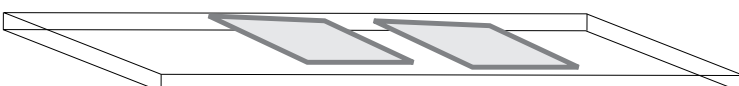
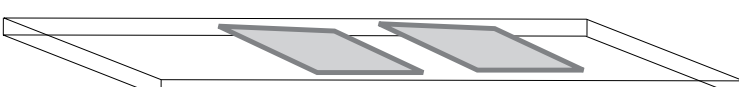

Facts about yogurt available free of charge from the National Dairy Council, 5–7 John Prince's Street, London W1M 0AP has more information about the commercial production and microbiology of yoghurt.



Yoghurt with another difference



Gram's stain

- 1 
Remove grease from slide
- 2 
Mark out 2 x 1cm² squares
- 3 
Syringe yoghurt suspension into squares
- 4 
Dry slide over Bunsen burner flame
- 5 
Apply Gram's stain (see right)

Cover slide with **crystal violet** solution and stain for 30 seconds. Rinse stain off with tap water or distilled water from a wash bottle.

Cover slide with **iodine solution** and stain for 30 seconds. Rinse stain off with tap water or distilled water from a wash bottle.

Decolorize slide with **ethanol (IMS)** until the washings are a very pale violet. Rinse with tap water or distilled water from a wash bottle.

Counter stain with **safranin solution** for 2 minutes. Rinse stain off with tap water or distilled water from a wash bottle.

Blot or air dry. Examine under a microscope fitted with an oil immersion lens.