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Through a microbiologist's eyes: optimal time to add honey to tea

Preparation of liquid nutrient media, nutrient agar and agar plates

Culture media and bacterial cultures should be prepared by the teacher or an experienced laboratory technician before Activity 1. Ready-made liquid medium and pre-poured agar plates may also be used. The preparation procedure described below is based in part on guidance provided by the National Agency for Education.[1]

Materials and equipment

- LB nutrient-medium powder
- Agar powder
- Water
- Heat-resistant bottles and beakers
- Measuring cylinder and balance
- Sterile 90 mm Petri dishes
- Sterile test tubes or culture flask
- Sterile inoculating loop
- Non-pathogenic teaching strain
- Pressure cooker or autoclave
- Incubator (optional)
- Bunsen burner or spirit lamp
- Personal protective equipment and surface disinfectant

Calculating the required volume of medium

Nutrient agar medium

For a 90 mm Petri dish, prepare approximately 25 ml of solid nutrient medium per plate.

Let:

- H = number of honey types tested in Activity 1
- A = number of active honey types selected for Activity 2
- T = number of heating temperatures tested for each selected honey type

Activity 1

One Petri dish can contain:

- three wells for three honey samples
- one well for the sterile water negative control
- one well for the artificial honey analogue (AH)

The number of plates required per group is H ÷ 3, rounded up to the next whole number.

For example, if four honey types are tested, two plates are required:

- Plate 1: two honey samples + artificial honey analogue + sterile water control
- Plate 2: two honey sample + artificial honey analogue + sterile water control

Activity 2

A negative control is not required. One Petri dish can contain up to four honey samples heated to different temperatures. Use separate plates for each selected honey type.

The number of plates required is A × (T ÷ 4), rounded up to the next whole number.

For example, if two active honey types are tested at six temperatures, two plates are required for each honey type. That makes a total of 4 plates required (2 honey types × 2 plates).

Total volume

The total number of plates required per group is Activity 1 plates + Activity 2 plates.

The required volume of solid nutrient medium is the number of plates × 25 ml.

For example:

If four honey types are tested in Activity 1 and two active honey types are tested at six temperatures in Activity 2, you need:

- Activity 1: 2 plates
- Activity 2: 4 plates
- Total: 6 plates

6 × 25 ml = 150 ml of solid nutrient medium per group

Prepare approximately 10% extra medium to account for losses during heating and pouring.

Replicates

For more reliable results, repeat the complete test three times using separate plates.

For the example above:

6 plates × 3 replicates = 18 plates

18 × 25 ml = 450 ml of solid nutrient medium per group

Prepare an additional 10% for losses.

Liquid nutrient medium

Prepare approximately 0.5 ml of fresh bacterial liquid culture per group for each activity.

Therefore:

- Activity 1: 0.5 ml per group
- Activity 2: 0.5 ml per group

The total amount of liquid medium can be calculated as number of groups × 0.5 ml × 2 activities.

For example, a class of 20 students working in pairs forms 10 groups:

10 × 0.5 ml × 2 = 10 ml of liquid medium.

Prepare a small additional volume to allow for pipetting losses.

For the most reliable results, prepare a fresh bacterial culture before each activity. If this is not possible, keep the culture tightly closed at approximately 4°C and use it within 24 hours.

Preparation of liquid nutrient medium

Fresh bacterial liquid cultures are required for both Activity 1 and Activity 2. Prepare approximately 0.5 ml of liquid nutrient medium per group for each activity. It is best to prepare a fresh culture before each activity.

The total volume required can be calculated as number of groups × 0.5 ml × 2 activities.

For example, a class of 20 students working in pairs forms 10 groups: 10 groups × 0.5 ml × 2 = 10 ml of liquid nutrient medium.

Prepare a small additional volume to allow for pipetting losses.

Materials

For 25 ml media, you need:

- 0.75 g LB nutrient-medium powder
- Water to a final volume of 25 ml
- Optional: 0.5 g sucrose (can be omitted when only bacteria are being cultured)

Procedure

1. Weigh 0.75 g of LB powder and, if used, 0.5 g of sucrose.
2. Transfer the ingredients to a small heat-resistant beaker.
3. Add water to a final volume of 25 ml and stir until the ingredients have dissolved.
4. Transfer the medium to a heat-resistant bottle with a capacity of at least 75 ml, so that the liquid occupies no more than one third of the bottle volume.

5. Loosen the bottle cap slightly and cover it loosely with aluminium foil. Do not close the bottle tightly during sterilisation.
6. Add water to the pressure cooker. In the original protocol, approximately 500 ml of water was used. However, always use at least the minimum volume specified by the pressure-cooker manufacturer.
7. Place a rack or heat-resistant support inside the pressure cooker, if required, and position the loosely capped bottle upright. The bottle should not stand directly on the heated base unless this is permitted by the manufacturer.
8. Close the pressure cooker securely and check that the lid, seal and safety valves are clean and undamaged.
9. Heat the pressure cooker until operating pressure is reached. Once the required pressure has been reached, sterilise the medium for approximately 30–45 minutes.
10. Turn off the heat and allow the pressure cooker to cool naturally. Do not move or open it while it is hot or pressurised.
11. Wait until the pressure has completely returned to normal. This may take approximately 20–30 minutes or longer, depending on the equipment and volume.
12. Open the pressure cooker carefully, directing the lid away from the face and body, and remove the bottle using heat-resistant gloves.
13. Allow the sterile liquid medium to cool to room temperature before dispensing.
14. Working beside a Bunsen burner or spirit-lamp flame, dispense 0.5 ml of sterile liquid medium into one sterile test tube for each group. Open the bottle and test tubes only briefly and close or cover them immediately after dispensing.
15. Prepare one set of tubes for Activity 1 and a fresh set for Activity 2. If both activities are not carried out on the same day, store the remaining sterile medium in a tightly closed sterile container at approximately 4°C and dispense the second set shortly before use.

Storage note: Sterile liquid medium can be stored in a tightly closed sterile bottle or sterile test tubes at approximately 4°C for several days. Before use, allow it to reach room temperature and check that it remains clear. If the medium becomes cloudy, contains visible particles or shows any other signs of growth, it may be contaminated and should not be used.

Fresh culture note: For the most reliable results, prepare a fresh bacterial liquid culture before both Activity 1 and Activity 2. If immediate use is not possible, keep the inoculated tubes tightly closed at approximately 4°C and use them within 24 hours.

Autoclave option: If an autoclave is available, sterilise the medium using the laboratory's validated cycle, typically at 121°C for 15–20 minutes, following the manufacturer's instructions and local safety procedures.

Preparation and pouring of nutrient agar plates

Prepare the required volume of solid nutrient medium calculated above. The quantities below are given for 100 ml and should be adjusted proportionally.

Materials

For 100 ml of solid nutrient medium:

- 3 g LB nutrient-medium powder
- 2 g agar powder
- Water to a final volume of 100 ml
- Optional: 2 g sucrose (can be omitted when only bacteria are being cultured)

Procedure

1. Weigh the LB powder, agar and, if used, sucrose.
2. Transfer the LB powder and sucrose to a heat-resistant beaker.
3. Add water to the required final volume and stir until the LB powder and sucrose have dissolved.
4. Divide the prepared liquid medium between one or more heat-resistant bottles. The liquid should occupy no more than one third of each bottle's total volume.
5. Add the appropriate amount of agar directly to each bottle and gently mix to distribute it evenly. The agar may not dissolve completely before sterilisation.
6. Loosen the bottle cap slightly and cover it loosely with aluminium foil. Do not close the bottle tightly during sterilisation.

Safety note: Media sterilisation should be carried out only by a trained adult using equipment approved by the school or institution. An autoclave should be used where available. A pressure cooker should only be used if this method is permitted by local institutional safety procedures and must be operated strictly according to the manufacturer's instructions. Students should not operate pressurised sterilisation equipment.

7. Add water to the pressure cooker. In the original protocol, approximately 500 ml of water was used. However, always use at least the minimum volume specified by the pressure-cooker manufacturer.
8. Place a rack or heat-resistant support inside the pressure cooker, if required, and position the loosely capped bottle upright. The bottle should not stand directly on the heated base unless this is permitted by the manufacturer.
9. Close the pressure cooker securely and check that the lid, seal, and safety valves are clean and undamaged.

10. Heat the pressure cooker until operating pressure is reached. Once the required pressure has been reached, sterilise the medium for approximately 30–45 minutes.
11. Turn off the heat and allow the pressure cooker to cool naturally. Do not move or open it while it is hot or pressurised.
12. Wait until the pressure has completely returned to normal. This may take approximately 20–30 minutes or longer, depending on the equipment and volume.
13. Open the lid carefully, directing it away from your face and body to avoid escaping steam. Remove the bottle using heat-resistant gloves.
14. Gently swirl the bottle to distribute the agar evenly. Do not shake, as this may create bubbles.
15. Allow the medium to cool to approximately 50°C. It should remain liquid but be cool enough to handle safely.
16. Work on a clean, disinfected surface within the aseptic zone near the Bunsen burner flame or spirit lamp. Keep flammable disinfectants away from the flame.
17. Open each sterile Petri dish only slightly and pour approximately 25 ml of medium into the dish.
18. Close the lid immediately. If necessary, gently rotate the closed dish so that the medium covers the base evenly.
19. Stack the plates while the agar solidifies to help reduce condensation on the lids.
20. Once the agar has set, store the plates upside down, with the agar side facing upwards.
21. Prepared plates can be stored for several days at approximately 4°C in sealed packaging or a clean closed container. Allow them to reach room temperature before use.

Note: Commercial sterile plastic Petri dishes must not be heat sterilised. Reusable glass Petri dishes, however, can be sterilised in a pressure cooker or autoclave, or by dry heat at 160–170°C for approximately 2 hours.

Autoclave option: If an autoclave is available, sterilise the medium using the laboratory's validated cycle, typically at 121°C for 15–20 minutes, following the manufacturer's instructions and local safety procedures.

Sterilisation of equipment:

Reusable glassware and heat-resistant metal tools should be sterilised before use.

- Glass test tubes, glass Pasteur pipettes, and reusable glass Petri dishes may be sterilised in a pressure cooker or autoclave. Keep test tubes uncapped or loosely capped and cover containers loosely with aluminium foil.
- Metal forceps, inoculating loops and spreaders may be sterilised in a flame before and between uses. Allow them to cool before touching cultures or agar.

- Alternatively, heat-resistant metal tools may be disinfected with 70% ethanol and allowed to dry completely before use. Keep ethanol away from open flames.
- Autoclavable pipette tips may be sterilised in an autoclave or pressure cooker according to the manufacturer's instructions. Keep them in a suitable tip box or heat-resistant container and cover loosely during sterilisation.
- Sterile disposable pipette tips, swabs, loops, and plastic Petri dishes should be used directly and must not be heat sterilised.
- After sterilisation, keep all equipment covered or closed until use to minimise contamination.

References

[1] Preparation of nutrient media for growing microorganisms: https://www.scribd.com/document/529433106/206-Lab-Ex-3-media-Preparation