Bacterial growth, plant antimicrobials and drug testing

Conditions for bacterial growth, antimicrobial substances from plants, aseptic technique, and the history and stages of drug testing.

Conditions for bacterial growth

Bacteria need:

- a **carbon source** (e.g. glucose) and a **nitrogen source** (e.g. amino acids or ammonium ions) in a **nutrient medium** (broth or agar),
- **minerals and growth factors**,
- a suitable **temperature** (about 25–37 °C), so enzymes work at their optimum (human pathogens grow best at 37 °C, but cultures in schools are kept at about 25 °C to avoid growing pathogens),
- a suitable **pH**,
- **water**, and **oxygen** (or its absence, for anaerobes).

Population growth is rapid because bacteria reproduce by **binary fission**, and a generation can take as little as 20 minutes.

Antimicrobial substances from plants

Many plants make chemicals that protect them against bacteria, fungi and herbivores. Some are used as **medicines** with antimicrobial and other therapeutic properties.

- **Garlic** (allicin), **tea tree oil**, **thyme** (thymol), **cloves**, **honey**.
- **Digitalis** from foxglove: a heart drug (digoxin).
- **Willow bark**: the source of aspirin.
- **Quinine** from cinchona bark treats malaria.

Their uses: as antibiotics, painkillers or heart drugs, and as leads for **new drugs**. A reason to conserve plant **biodiversity** is that the source of future medicines may be lost.

Core Practical 9: antimicrobial properties of plants

Aseptic technique prevents contamination of the culture and the environment and protects the worker:

1. Disinfect the work surface, and wash hands.
2. Pass the neck of the bottle and the **inoculating loop** through a **Bunsen flame** to sterilise it (the loop is flamed until red hot and cooled).

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3. Open the lid of a Petri dish **only enough** to work, and keep it close to the flame (an updraught keeps away airborne microbes).
4. **Spread** the bacteria evenly over the agar with a sterile spreader.
5. Put **discs** of filter paper soaked in plant extracts (and a **control** disc soaked in sterile water or solvent, and a disc with a known antibiotic as a positive control) on the agar.
6. **Tape** the lid on with two or four pieces of tape (not sealed all round, so that oxygen enters and anaerobic pathogens cannot grow), **invert** the dish, and incubate at **25 °C** (not at 37 °C) for 24–48 hours.
7. Measure the diameter of the **zone of inhibition** (the clear area where bacteria did not grow), and calculate the area, $A = \pi r^2$.

A larger zone means a stronger antimicrobial effect (although it also depends on **diffusion** of the extract through the agar). Dispose of used plates by **autoclaving**.

> **The reasons for the safety rules: do not culture at 37 °C (which would favour human pathogens), do not fully seal the dish (it would allow anaerobic pathogens), and keep the dish taped and never opened after incubation.**

Drug testing

Historical methods

William Withering (1785) investigated **foxglove** (digitalis) as a treatment for dropsy (heart failure). He tested **different doses** on patients, recorded the effects and found that the correct dose varied, but his early "digitalis soup" was an unreliable mixture, and there were no controls.

Modern protocols

- **Pre-clinical testing**: on cells in culture, computer models and animals, to check safety and effect.
- **Three phases of clinical trials**:
  1. **Phase 1**: a small group of **healthy volunteers**, to test **safety** and the dose.
  2. **Phase 2**: a larger group of **patients** to test whether it works and to find side effects.
  3. **Phase 3**: a **large** number of patients, compared with the best existing treatment or a placebo.
- **Placebo**: a harmless substance that looks identical to the drug, to separate real effects from psychological ones (the **placebo effect**).
- **Double blind trials**: **neither** the patients **nor** the researchers who assess them know who has the drug and who has the placebo, to avoid bias. Patients are **randomly** allocated.

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- Results are checked for **statistical significance**, then **peer reviewed** and published, and regulatory bodies decide whether to license the drug. After licensing, side effects continue to be monitored.

NOTE

Ethical considerations: informed **consent**, the right to withdraw, the risk to volunteers, use of animals, and whether it is right to give a placebo to patients who need treatment.

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