Why do they put mint in toothpaste? Would garlic be better?

Plants are susceptible to infection by bacteria and fungi; they do everything to repel such attacks. Several plants are known to, or thought to destroy or inhibit the growth of certain bacteria. A plant with this property is known as antibacterial. Chemicals in their cells are toxic to bacteria or interfere with their metabolism in some other way. You can probably guess why there is mint in toothpaste, but would garlic be better? Mint may numb our gums but is it lethal to bacteria or insects? In this activity you will investigate whether two plants contain antibacterial chemicals and their effectiveness by looking at the growth of bacteria on agar plates.

Before you start, read through the procedure and suggest what you might expect to observe on the plates. Decide how you would take precise measurements to enable you to make reliable conclusions from the data.

Safety

Use sterile techniques. Do not open Petri dishes used for growing microorganisms. Do not dispose of them before they are autoclaved. Methylated spirits is toxic and highly flammable and because of the latter hazard should not be used while naked flames are in use – as they will be in the preparation and pouring of agar plates.

You will need

- Agar plate seeded with bacteria
- Plant material (garlic cloves and mint leaves)
- Pestle and mortar
- 10 cm^3 industrial methylated spirits
- Pipette (sterile)
- Paper discs (e.g. Whatman antibiotic assay paper discs)
- Sterile Petri dish
- Sterile forceps
- Tape
- Marker pen
- Incubator set at 25 °C

Procedure

1 Agar plates seeded with suitable bacteria need to be prepared. This may have been done for you in advance; if not, follow the instructions on the sheet 'Pouring agar plates'.

2 Obtain a plant extract by crushing 3 g of plant material with 10 cm^3 of industrial methylated spirit, and shake it from time to time for 10 min. The advantage of using methylated spirits instead of water is that it kills any bacteria that might otherwise contaminate the extract.

3 Pipette 0.1 cm^3 of extract onto a sterile 13 mm Whatman antibiotic assay paper disc. (If these are not available, discs cut from new filter paper using a hole punch can be used.)

4 Let the paper discs dry for 10 min on open sterile Petri dishes.

5 Repeat steps 1 to 4 for other plants, making separate test discs for each extract.

6 Decide within your group what the "suitable control" should be. Check with your teacher/lecturer before proceeding. Use sterile forceps to place the disc onto the bacterial plate together with a suitable control per plate. Three test discs and a control can be placed on a single Petri dish. Ensure that you can distinguish between the different discs.

7 Close the Petri dish and tape it as shown in Figure 1. Do not tape all round the dish because this can lead to the growth of anaerobic bacteria, some of which may be harmful. Make sure your name, the date and plant and bacteria used are recorded on the plate.

8 Incubate the plates for 24 hours at 25 °C.

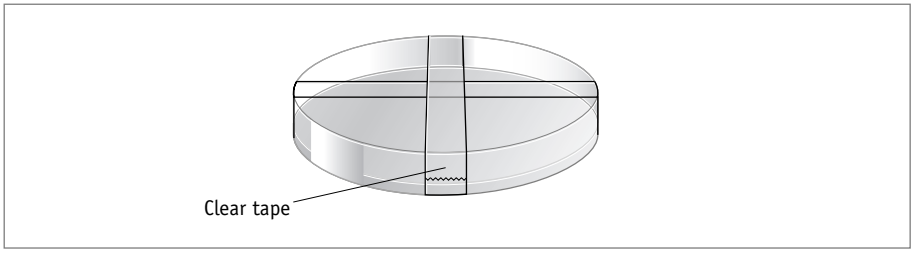
9 Observe the plates without opening them. Bacterial growth on an agar plate looks cloudy. Make any appropriate measurements that will enable you to compare the antibacterial properties of the different plant extracts.

10 Do not throw the plates in the bin. (The unopened Petri dishes will need to be autoclaved before disposal in the dustbin.)

11 Wash your hands thoroughly with soap and water after completing the practical.

12 Present your results in the most appropriate way.

13 Write up your experiment. Make sure you discuss safety precautions taken. Explain any patterns in the data using evidence from the data and your own biological knowledge. How reliable is your conclusion? Comment on how you ensured that the results obtained in this experiment were valid; how were precise measurements made? Comment on how you could have made your results more reliable.



▲ **Figure 1** A convenient way of taping a Petri dish without allowing anaerobic conditions to develop.

Pouring agar plates

Safety

Do not do this if methylated spirits is in use as part of this activity.

Aseptic techniques should be used throughout to avoid contamination.

1. Collect a bottle or test tube containing 15 cm^3 of sterile nutrient agar.
2. Melt the agar by placing the bottle or tube in a hot water bath (agar melts at 97 °C). If the bottle has a screw cap it should be loosened to allow air to escape.
3. Once all the agar has melted remove the bottle. You will need to use a cloth to do this. Allow the agar to cool to about 50 °C, a temperature at which you can handle the bottle. The agar will start to solidify at about 42 °C. Take care not to let it cool too much or it will set as you pour it into the Petri dish.
4. Pipette 1 ml of bacterial broth into a sterile Petri dish using an aseptic technique. The lid of the Petri dish should only be lifted enough to allow entry of the pipette. See the figure below.
5. Pour the 15 ml of molten agar into the Petri dish. Gently push the plate back and forth, N-S, NE-SW and NW-SE to mix the bacteria with the agar and allow the agar to set.
6. Please note: *It is essential* that the plates are used for the investigation an hour or so after the agar has set, otherwise once the bacteria have started to grow they will be unaffected by the antimicrobial agent.