WHY DO THEY PUT MINT IN TOOTHPASTE? WOULD GARLIC BE BETTER?

Purpose

To investigate the antibacterial properties of plants.

To develop practical skills.

YOU NEED	
• Agar plate seeded with bacteria • Plant material (garlic cloves and mint leaves) • Pestle and mortar • 10 cm^3 industrial methylated spirit • Pipette (sterile) • Paper discs (for example, Whatman antibiotic assay paper discs)	• Sterile Petri dish • Sterile forceps • Tape • Marker pen • Incubator set at 25 °C

SAFETY

Wear eye protection, lab coats and disposable gloves.

Industrial methylated spirit is harmful and highly flammable and because of the latter hazard should not be used while naked flames are in use. See CLEAPSS Hazcard 40A for details. Do not use if the preparation and pouring of agar plates is being done elsewhere in the lab.

Use aseptic techniques. Do not open Petri dishes containing growing microorganisms. Only dispose of used Petri dishes after they have been autoclaved. See CLEAPSS Student Safety Sheet 1 and Practical Skills Support Sheet 11 for further details.

Wash your hands thoroughly after handling plant material or growth media.

Antibacterial chemicals

Plants are susceptible to infection by bacteria and fungi; they do everything they can 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? In this activity you will investigate if 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 valid conclusions from the data about whether or not the plant extracts have antimicrobial properties and if they are equally effective.

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 page 3. The Practical Skills Support has a sheet on plate pouring and aseptic technique.

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 minutes.

3 Pipette 0.1 cm^3 of extract onto a sterile 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 minutes 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 a 'suitable control' should be. Check with your teacher/lecturer before proceeding.

7 Use sterile forceps to place the test discs onto the bacterial plate together with the 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 by marking the underside of the Petri dish.

8 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, the plant and bacteria used are recorded on the plate.

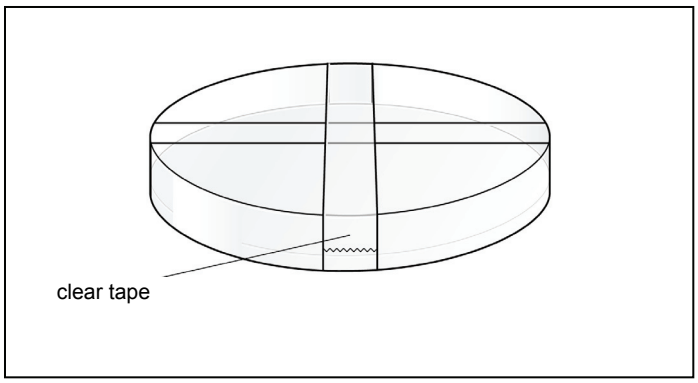

Figure 1 A convenient way of taping a Petri dish without allowing anaerobic conditions to develop.

9 Incubate the plates for 24 hours at 25 °C (longer incubation times may be required for the growth to be visible, so it may be necessary to observe them at intervals over several days).

10 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.

11 Return plates to your teacher to be autoclaved to kill bacteria before disposal of the plates.

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

Analysis and interpretation of data

Present your results in the most appropriate way. See Maths and Stats Support Sheet 1 – presenting data – tables, in the support section of SNAB Online. Remember to record a suitable number of significant figures in measured and calculated values. If you have repeated measurements use these to comment on the significance of your results. See Maths and Stats Support Sheet 9 for an introduction to statistical tests.

Conclusion and evaluation

In the write up of your experiment, making sure your report includes:

- a clear conclusion to your work that explains any patterns in the data using evidence from the data and your own biological knowledge
- comments on how valid your conclusion is
- comments on the accuracy and precision of the results obtained in this experiment
- comments on whether or not the outcome of your work was as you expected. If it wasn't, try to explain why not.
- discussion about any safety precautions you took during the experiment
- descriptions of any modifications you made to the procedure and how the experiment could be improved.

Pouring agar plates

SAFETY
Wear eye protection and a lab coat. *Do not do this procedure if methylated spirit is in use.* *Aseptic techniques should be used throughout to avoid contamination.*

YOU NEED	
• 15 cm^3 of sterile agar in an agar bottle or test tube • Beaker into which the agar bottle will fit	• Sterile Petri dish • 1 cm^3 sterile pipette

Procedure

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 cm^3 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 Figure 2 below.

5 Pour the 15 cm^3 of molten agar into the Petri dish and replace the lid. 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.

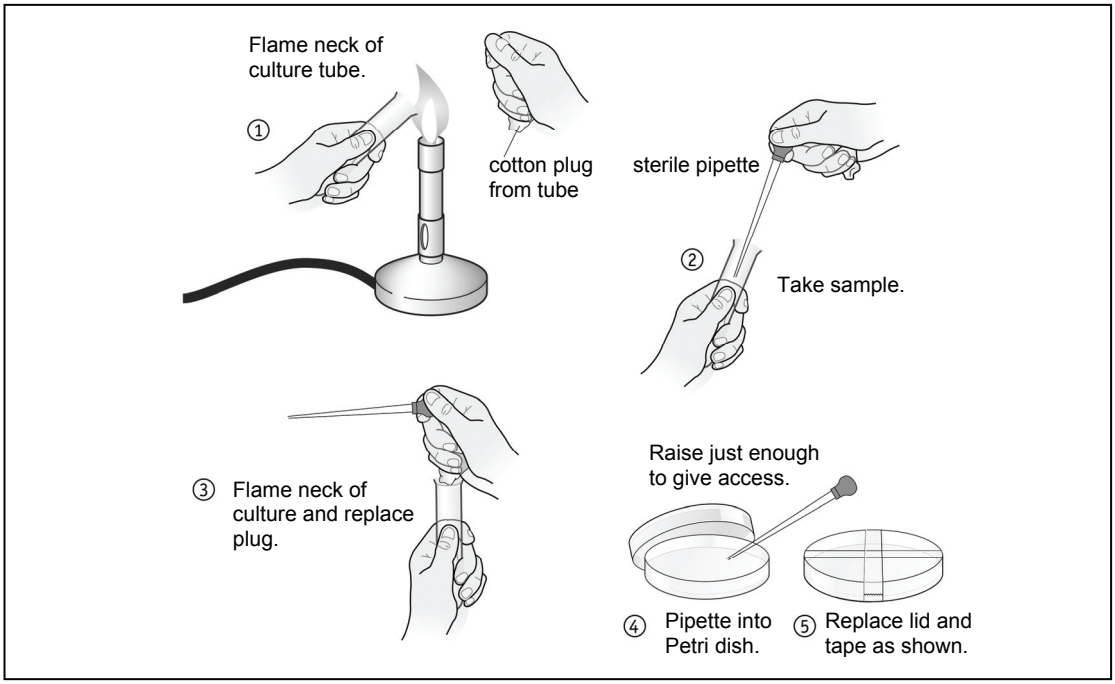

Figure 2 Aseptic techniques.

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Ensure eye protection and lab coats are worn throughout.

Teachers must be familiar with the procedures and techniques used in this activity beforehand. Inexperienced staff will require training. Students will need to be told to observe basic hygiene rules and be taught aseptic techniques. Particular care needs to be taken when cleaning up spills and disposing of cultures. Students should not be involved in these aspects of the activity, but should report any problems to the teachers. See CLEAPSS Student Safety Sheet 1 and the microbiology section in the CLEAPSS biology handbook for further details.

Ensure students do not open the incubated plates.

Staphylococcus albus *has been known to infect individuals who are debilitated or taking immuno-specific drugs. Ensure that no one using it is at such a risk.*

Ensure industrial methylated spirit (harmful, highly flammable, see CLEAPSS Hazcard 40A) is not in use when agar plates are being prepared.

Notes on the procedure and developing practical skills

The students could just test mint and garlic to address the questions in the title of the activity or they could bring in a wide range of different plants to test.

There has been research into the antibacterial properties of spices used in cooking. In 1998, two scientists, Jennifer Billing and Paul Sherman, investigated the apparent connection between hot countries and the use of spices in cooking. After examining 43 spice plants and testing their antibacterial properties, a top ten list of the most antibacterial seasonings was calculated: garlic, onion, allspice, oregano, thyme, cinnamon, tarragon, cumin, cloves, lemongrass.

Several hypotheses have been put forward to explain the use of spices. Spices help to flavour food and may in the past have been used to disguise the bad taste of spoiled food.

Hot, spicy food causes the body to sweat and thus helps to cool a person down by evaporation. Spices provide micronutrients. However, these hypotheses seem unconvincing compared with the survival value of adding spices that inhibit the growth of food-borne bacteria.

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. Therefore it may be more suitable for students to prepare their own plates. The advantage of using methylated spirit instead of water is that it kills any bacteria that might otherwise contaminate the extract.

This is a Core Practical: it is used to develop students' microbial aseptic technique and investigate skills. The Student Sheet is structured with the development of these skills in mind. Safety is an important aspect of this experiment and the need to take precautions when undertaking any microbiology should be stressed. Guidance on microbiology is available in the ASE publication *Topics in Safety*, 3rd edition (2001). Also useful is *Microbiology: an HMI Guide for Schools and FE* (1990) HMSO.

The worksheet does not say what measurements students should make when they observe their plates. They should be encouraged to think about ensuring that they have valid results by making suitable and precise measurements. A clear area where the bacterial growth has been inhibited should surround each of the discs. The simplest measurement would be to use a ruler and measure the diameter of the cleared area. It is straightforward to compare the results of different treatments if the clear areas are perfect circles. If the diameter varies, one possibility is to measure at the widest point. For a more precise measurement, the area of the clear zone would have to be determined. Students could suggest possible ways of doing this. Results could be shared among students to provide repeated measurements and complete an appropriate statistical test.

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Ensure industrial methylated spirit (harmful, highly flammable) is not in use when agar plates are being prepared.

Requirements per student or group of students	Notes
Agar plate seeded with bacteria (Suggested organisms are: *Bacillus subtilis*, *Escherichia coli* (strain K12) and *Staphylococcus albus*.)	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. Therefore it may be more suitable for students to prepare their own plates. Notes on the preparation of the agar plates are provided on the additional sheet *Pouring agar plates*. Bacteria can all be obtained from biological suppliers or NCBE. Notes on the preparation of the broth culture are given on page 2.
Plant material (garlic and mint)	Garlic and mint are suggested because of the questions posed in the title of the activity, but a range of different plant material could be tested.
Pestle and mortar	
10 cm^3 industrial methylated spirit	Highly flammable and toxic. See CLEAPSS Hazcard 40A.
Pipette (sterile)	
Paper discs (e.g. Whatman antibiotic assay paper discs)	New filter paper cut using a hole punch could be used. Antibiotic assay discs are sterile. They do not contain antibiotic but are coated in the solution to be tested.
Sterile Petri dish	
Sterile forceps	
Tape	
Marker pen	
Incubator set at 25 °C	

Making a broth culture

It is likely that the bacteria will be sent as slope cultures. To make a broth culture the following procedure can be used.

1 Flame the neck of the culture. Allow to cool in the air for a few seconds after flaming. Scrape off a small amount of bacteria from the surface using a sterile inoculation loop. Transfer this to a batch of sterile nutrient broth, having first flamed the neck of the broth container.

2 Incubate the broth until it becomes turbid. A suitable temperature is 25 °C. The temperature must not exceed 30 °C. This might take 2–3 days with some bacteria. *E. coli* grows fastest.

3 To make up the agar plates, use about 1 cm^3 of bacterial broth for each sterile Petri dish.

Guidance on microbiology is available in the following publications: ASE (2001) *Topics in Safety,* 3rd edition, Hatfield: Association of Science Education and DES (1990) *Microbiology: an HMI Guide for schools and FE*, London: HMSO.

Notes