22 - Fungi

General precautions when growing micro-organisms

Aseptic or **asepsis** means that measures are taken to exclude unwanted micro-organisms.

Sterile means that all micro-organisms are destroyed, i.e. there is nothing living.

In general you should assume that all micro-organisms are potentially harmful, unless it is stated or proven otherwise.

Aseptic techniques

Aseptic techniques involve the creation of a germ-free environment in as far as is possible. Aseptic methods include the following procedures:

1. Wash your hands before and after each experiment
2. Wash the bench with disinfectant before and after each experiment
3. Do not put fingers, food, drink or equipment in or near your mouth
4. Keep all containers closed where possible
5. (i) Open all containers for the shortest possible time and (ii) open lids the shortest possible distance (minimal opening)
6. When micro-organisms are in a petri dish, seal the dish with adhesive tape.

Sterile techniques

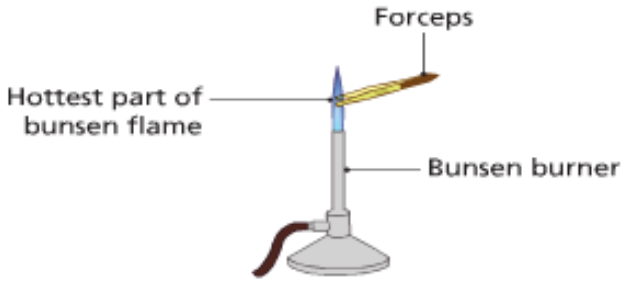
1. Sterilise all equipment before use or use equipment that is already sterile. This can be done by placing the equipment (except plastic) in a pressure cooker (or autoclave) at 120°C for 15 minutes or by placing it in an oven at 160°C for an hour.
2. Pass the neck of test tubes, needles or loops through the flame of a bunsen burner.
3. Flame all test tube necks, needles and loops again after they are used.
4. At the end of the experiment immerse all equipment and cultures in sterilising fluid.

The material can then be put in a dustbin or, in the case of glassware and metal, cleaned and reused as usual.

Activity 16 To investigate the growth of leaf yeast using agar plates

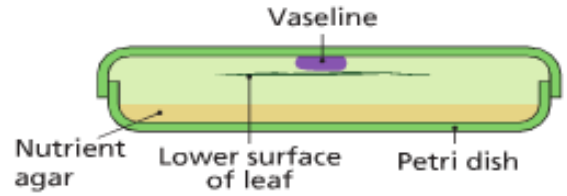
Micro-organisms are widely found in nature. This activity shows that, although they are not visible to the naked eye, leaves have many yeasts growing on their surfaces. These yeasts do not harm the leaves.

1. Cut a small branch containing some leaves from an outdoor plant – privet, ash or sycamore leaves are ideal. (These will be tested for the presence of leaf yeasts.)
2. Wash your hands with an aseptic soap solution. (This reduces the chance of micro-organisms being on your hands.)
3. Wash the bench or worktop with disinfectant. (Again this eliminates micro-organisms.)
4. Sterilise a forceps by heating it in the flame of a bunsen burner for a few seconds. (This means there will be no micro-organisms on the forceps.)
5. Obtain two sterile petri dishes containing prepared sterile nutrient agar. (Agar is a material derived from seaweed. It is used to form a solid growth medium. The nutrient agar provides food for micro-organisms to grow.)
6. Use the forceps to pick up one of the leaves, which should be small enough to fit across a petri dish. (This prevents micro-organisms getting onto the leaf from your hands.) Alternatively, for large leaves, you may flame a cork borer or scissors, allow it to cool and use it to cut a number of leaf discs.



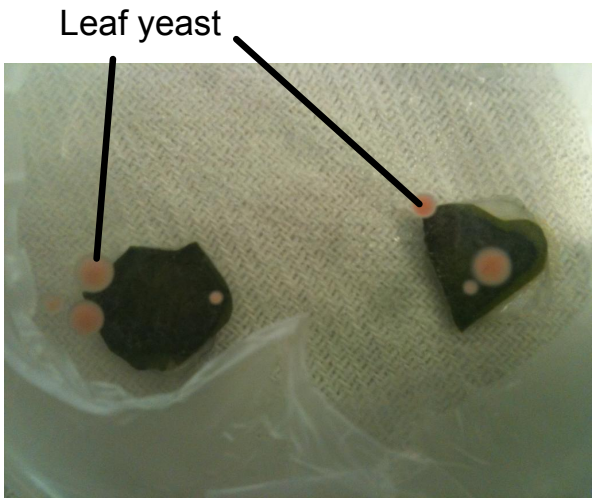
21.15 *Flaming a forceps*

7. Place a small spot of petroleum jelly (such as Vaseline) on the inside lid of the petri dish. (This will be used to attach the leaf to the lid of the petri dish.)
8. Reflame the forceps and allow it to cool.
9. Barely open the lid of one of the petri dishes in terms of (a) the distance it is opened and (b) the time for which it is opened. Use the forceps to attach the **upper** surface of the leaf to the lid of the petri dish. Make sure that the leaf does not touch the agar. Close the lid of the petri dish. The lower surface of the leaf is now facing down onto the agar. (There are more micro-organisms on the lower surface of the leaf than on the upper surface. The upper surface is covered by a cuticle which prevents the growth of micro-organisms. Leaf yeasts can expel their spores onto the surface of the agar.)



21.16 *Leaf attached to lid of petri dish*

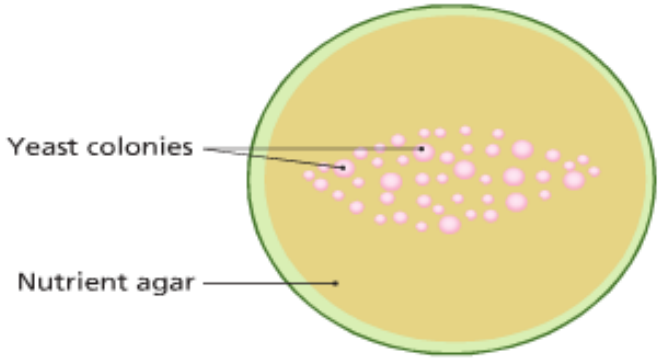
10. Reflame the forceps. (This will kill any micro-organisms on it.)
11. Seal a sterile nutrient agar petri dish containing no leaf. (This dish will act as a control or comparison. The only difference between the two petri dishes is that one contains a leaf and the other does not.)
12. Seal the petri dishes with tape or parafilm. (This prevents them from opening by accident.)
13. Label the petri dishes on the undersurface with a marker. (This allows the dishes to be identified, without further blocking the view of the agar surface.)
14. Leave the petri dishes at room temperature or in an oven or incubator at 25°C. (Leaf yeasts grow well at room temperature, but a higher temperature will speed up their growth.)
15. The dishes should be incubated upside down. (This prevents condensation forming on the lids.)
16. Observe the surfaces of the agar each day for three or four days (to see if any yeast colonies are forming.)



17. The expected results are:

- The dish with the leaf should show pink yeast colonies on the surface of the agar. These colonies may form a pattern similar to the shape of the leaf. Very few other micro-organisms will grow on the agar, unless part of the leaf is touching the agar. (The yeast can expel spores from a distance onto the agar; most other micro-organisms cannot grow across the space.)
- There should be no growth in the control dish. (This shows that the yeasts did not arise from any other source except the leaf.)

The growth of leaf yeasts is inhibited by air pollution. If the leaves are collected from a location with polluted air (such as a town or city) there may be few, or no, yeasts on the agar.



21.18 *Leaf yeast colonies growing on agar*

18. At the end of the experiment dispose of the agar and yeasts by sterilising it in an autoclave or pressure cooker for 15 minutes. Alternatively it can be immersed in sterilising fluid (such as Milton) and then put in a bin.