MiSAC*activities* 4

PRODUCED BY THE **MICROBIOLOGY IN SCHOOLS ADVISORY COMMITTEE**

Microbes on plant leaves

The microflora of plants

Some microbes found on plants have detrimental effects on the plant by causing well-known diseases that lead to serious economic losses of crops, e.g. soft rot of vegetables caused by *Erwinia* (a bacterium) and damping-off of seedlings by *Pythium* (a fungus). Also, certain species of *Pseudomonas* (a bacterium) serve as nuclei for the formation of ice crystals that cause damage to leaves. However, there are also many microbes on healthy leaves which cause no harm and play key roles in the commercially important processes of composting and ensilage.

The aims of this activity are (1) to show the presence of bacteria and fungi (i.e. yeasts and moulds) on the surfaces of healthy leaves; and to provide guidance on the contrasting principles of (2) composting and (3) ensilage.

1. FINDING MICROBES ON LEAVES

Materials (per group)

- 2 nutrient agar plates
- 2 malt extract agar plates
- 1 sterile Petri dish
- scissors
- forceps
- glass rod
- marker pen
- adhesive tape
- hand lens x10 magnification

Notes

- Flat, flexible leaves are easier to use than curved, tough ones.
- Students should devise a procedure which avoids contamination of the leaf surfaces from fingers and protects the agar culture media from contamination from the air.
- pH values of the culture media are: nutrient agar 7.4; malt extract agar 5.4.

Procedure

1. Choose a flower, shrub or tree, identify it, collect some healthy leaves and record their position on the plant.
2. Put the leaves into empty sterile Petri dishes and, if necessary, cut them into portions no larger than half of the dish using scissors and forceps.
3. Place a leaf with the upper surface down onto a nutrient agar (NA) plate, press firmly and discard the leaf.
4. Repeat the procedure with another NA plate and using the lower surface of another leaf from the same plant.
5. Repeat steps 3 and 4, pressing the leaves onto malt extract agar (MA) plates.
6. Label and tape the plates. Incubate them base uppermost at room temperature or 25^{o}C. Growth will begin to appear after 2 days.

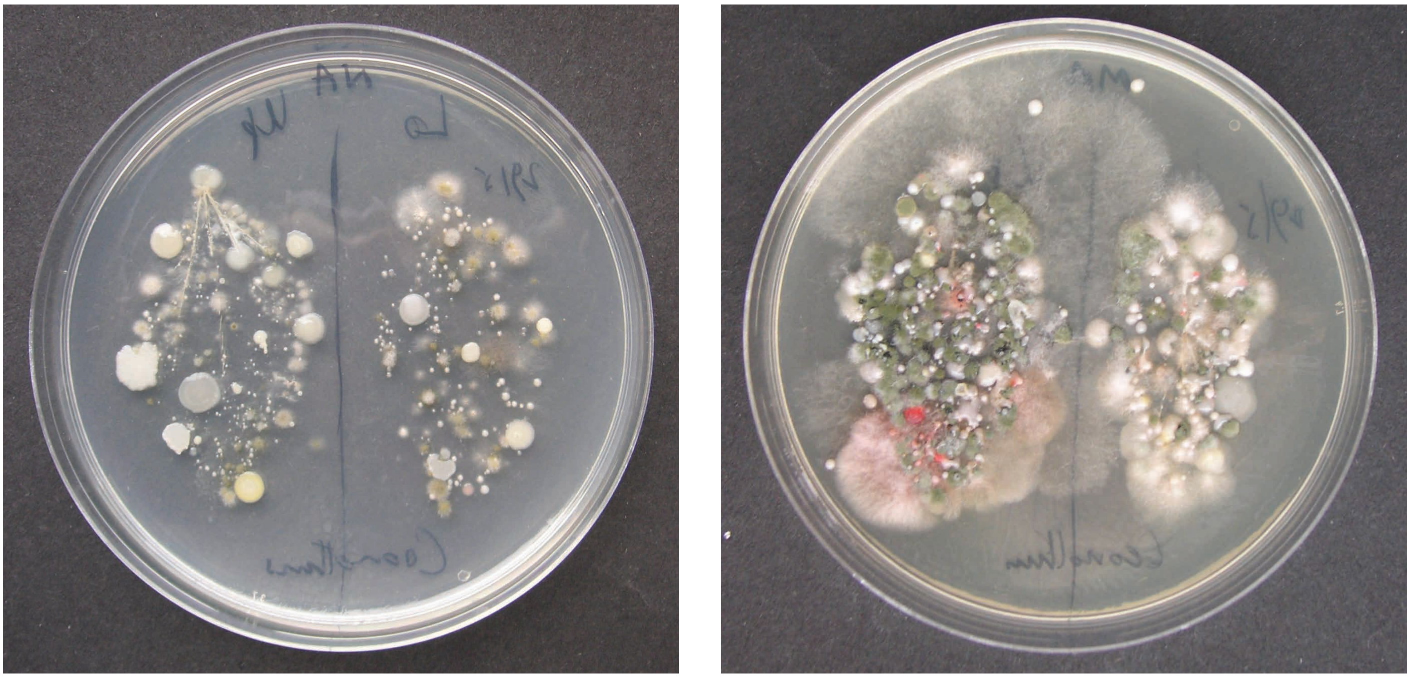
Next lesson

Without removing the lids of the Petri dishes*, examine the NA and MA plates by eye and with a hand lens for the presence of bacteria, yeast and moulds. Compare the appearance of the two media. Colonies of bacteria and yeasts appear as very small, circular structures after 1 day which after 2-3 days grow to a few millimetres in diameter and are shiny and off-white, yellow or red. In contrast, moulds appear after 2-3 days as very small knots of branched strands (hyphae) visible only through a x10 hand lens but on further incubation develop into colonies several centimetres in diameter with aerial spores on the surface which give the growth a dull, fluffy appearance, commonly green, black or white. See over page for photographs of a typical outcome.

***Health & safety note**: Incubation must not be continued beyond about 10 days because moulds produce large numbers of spores which if accidentally released to the air and inhaled are a potential health hazard. All plates must be autoclaved before disposal.

Extension activities

- Estimate the numbers of microbes per unit area of the upper and lower surfaces of a leaf, e.g. per cm^2.
- Compare the types and numbers of microbes on leaves taken from different parts of the same plant.



Leaf impressions from upper and lower surfaces of Ceanothus *on nutrient agar (left) and malt extract agar (right) media after incubation at room temperature for 4 days.*

2. GARDEN COMPOSTING

Composting is the controlled degradation of organic matter by biological oxidation to produce humus, a stable product that improves soil condition and enhances crop production. In consequence, the amount of waste disposed to landfill is reduced thereby making a contribution to controlling climate change. The process is the basis of the familiar garden compost heap. It is also done on a commercial scale.

The process requires the presence of microbes and the right conditions of nutrients, aeration, temperature and moisture for their growth. The relevant microbes occur naturally on household and garden waste which also provide the necessary nutrients, mainly carbon from 'browns' (e.g. paper, cardboard, fallen leaves, twigs) and nitrogen from 'greens' (e.g. weeds, lawn cuttings, vegetable waste). The aim is for a 1:1 mixture of 'browns' and 'greens'. The proportions can be changed if the material becomes too wet or dry.

The insulation properties of the mass of waste material contain excess energy released initially by respiring plant material and then by growth of the microbes, causing the temperature to rise to about 50°C, sometimes even to 60-70°C. This allows the dormant thermophilic (i.e. heat loving) microbes also naturally present to start growing and continue the aerobic degradation process. The process of natural composting takes 6-9 months; commercial processes are accomplished more quickly.

3. ENSILAGE

Silage is an important source of food for farm animals. It is made by a process known as ensilage by which specially-grown grass, maize and other seasonal fodder crops, which would become spoiled and unuseable during storage, are converted to a stable form which can be fed to animals throughout the year.

The process is a type of fermentation, i.e. it takes place anaerobically (in the absence of air), by lactic acid bacteria, e.g. *Lactobacillus*, which occur naturally on crops in small numbers and, much more importantly, also accumulate on harvesting machinery from where they provide the major part of the inoculum for the process. They ferment plant sugars (mainly pentoses) to lactic acid by the same process that converts milk into yoghurt and certain cheeses. The resulting low pH value reduces growth of other naturally-occurring microbes which can cause spoilage of the product and make animals refuse to eat it or become ill.

The major features of the technology of ensilage are (1) aiding the release of plant sugars by chopping the crop and increasing the amount of fermentable sugars by adding molasses, (2) controlling moisture content by allowing the freshly-cut crop to wilt on the field, and (3) providing anaerobic conditions by putting the harvested crop either in large, tall vessels (silos) or large covered heaps, or wrapping it in bales; depletion of oxygen is aided by the natural respiration processes of the plant material.