Leaving Cert Biology

Experiments

Investigate The Growth Of Leaf Yeast Using Agar Plates and Controls

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Materials/Equipment

Fresh leaves (e.g. common ash)
2 Sterile malt agar plates
Petroleum jelly
Disinfectant
Thermometer
Incubator (18℃ – 20℃)
Cork borer (approx. 1 cm diameter)
Chopping board
Bunsen burner
Matches/lighter
Marker/labels
Forceps
Paper towels
Masking tape/parafilm

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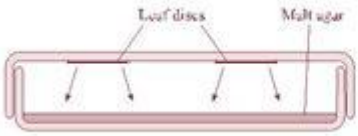
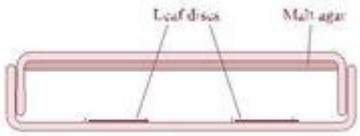


First 24 hours - leaf discs suspended over agar. Spores drop onto the agar.

Fig. 1



After 24 hours - invert the plates and incubate upside down for 3 days. Yeasts grow on the agar.

Fig. 2

Procedure

1. Familiarise yourself with all procedures before starting.
2. Swab the laboratory bench with disinfectant.
3. Seal one sterile malt agar plate and label as ‘control’. Initial and date.
4. Flame the cork borer and allow to cool.
5. Cut five discs from the leaves using the sterilised cork borer.
6. Take the second plate and place it upside down on the bench. (The malt agar is now in the top half.)
7. Lift the base of this plate (containing the agar) and place it open-side facing down on the bench. This will reduce potential contamination.
8. Flame the forceps to sterilise it and allow to cool.
9. Using the forceps, smear five small amounts of petroleum jelly (well spaced) on the inside of the lid.
10. Clean the forceps and re-flame it.
11. Use the forceps to attach a leaf disc to each of the blobs of petroleum jelly.
12. Replace the agar-containing base of the plate in the lid.
13. Clean and re-flame the forceps.
14. Seal the plate and label as ‘experiment’. Initial and date.
15. Re-swab the bench with disinfectant.
16. Invert this plate so that the leaf discs are uppermost and over the malt agar (Fig. 1). Leave the plate like this for approximately 24 hours so that spores can drop onto the agar from the leaf discs.
17. After 24 hours invert this plate (Fig. 2).
18. Incubate both plates (experiment and control) upside down, at 18 °C – 20 °C, for 3 days.
19. Compare the experiment plate with the control plate. Record the result. Leaf yeasts *(Sporobolomyces roseus)* will grow as pink glistening colonies.
20. Replicate the investigation or cross reference your results with other

groups.

Result

Agar plate	Appearance of colonies
Control	
Experiment	

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE GROWTH OF LEAF YEAST USING AGAR PLATES AND CONTROLS

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Label the plates
Swab the bench
Use the cork borer
Place the base of the experiment plate upside down on the bench
Sterilise the forceps and the cork borer
Smear the petroleum jelly on the lid
Seal the plates
Invert the experiment plate for approximately 24 hours
Re-invert this plate for 3 days
Use the thermometer
Incubate the plates

Observation
Observe the appearance of the colonies if present
Observe any differences between the experiment and the control plates

Recording
Write up the procedure
Tabulate the results
Record the appearance of the experiment plate
Record the appearance of the control plate

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others
Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Use aseptic technique throughout
Clean up after the practical activity

Background information

Sporobolomyces roseus

This species is the most common of the leaf yeasts. It grows on both the upper and lower surfaces of plant leaves. Its presence is influenced by factors such as leaf exudates, light intensity and temperature. The age of the leaf and its position on the tree also play a part in determining the presence and abundance of the yeast. Furthermore, the type and amount of wax, the number and position of stomata on the leaf surface and the presence of epicuticular hair will also play a role. This species grows as pink colonies and its abundance has been used to monitor air quality. It is known as a 'mirror' yeast because it can forcibly discharge its spores which then grow on the agar forming a mirror image. It is thought that the leaf yeasts overwinter on grasses, especially on ryegrass and meadow fescue. These then provide the source of the initial populations in the Spring/Summer.

S. roseus has evolved efficient air uptake mechanisms and as a result, where the air contains poisonous pollutants, particularly sulfur dioxide, the number of leaf yeast colonies per disc is greatly reduced. Since leaf yeast cells have a rapid life cycle, changes in the leaf populations can be used to monitor short-term changes in air quality. It is not possible to count yeast cells directly on the leaf surface but an indirect measure of population density can be obtained by measuring the number of colonies that can be isolated on agar plates from a given area of the leaf. This would be difficult to do with most leaf-surface fungi but the fact that *S. roseus* shoots basidiospores into the air where they can be intercepted, means that relatively simple techniques can be used. The number of colonies will reflect the health of the yeast populations and also the quality of the air. Large-scale comparative studies, carried out by school children in several European countries, have established that the lowest numbers of leaf yeast colonies correlate well with higher levels of sulfur dioxide pollution. Eanna Ní Lamhna of An Foras Forbartha co-ordinated the Irish studies undertaken from 1982 to 1986. Secondary school students from the mid west, the south coast, Cork city and the east coast used lichens, leaf yeasts (from the underside of ash tree leaves), and the acidity of the rainfall to monitor the air quality. While the lichens reflected the air quality in the few years prior to the study, the results for the leaf yeast study depended on the air quality in the weeks prior to the investigation.

Common ash – *Fraxinus excelsio*r

The common ash is a large familiar tree with a long silvery stem. The 20 cm – 30 cm leaves are pinnate with 9 – 13 toothed oval leaflets arranged in pairs with a single one at the tip.

In April, the flowers appear before the leaves – the flowers are green in colour, are small and inconspicuous having neither a calyx nor a corolla.

In Autumn, the leaves turn a muddy brown or yellow colour and are shed in October. The ash tree is hardy enough to survive almost anywhere. In Winter, clusters of black velvety buds will help to identify the tree.

Advance preparation

- Collect fresh leaves.
- Set the incubator and check the temperature with a thermometer.
- Prepare/purchase malt agar plates.

Helpful hints

- Leaves from common ash, lilac, sycamore, red alder or hawthorn are generally suitable for use in this investigation. Ash leaves are particularly good as they are widely available, easy to identify and have good yeast populations. Ash trees can be found in parks and roadsides and also in hedges used to mark field boundaries. Clover leaves and cherry laurel leaves release cyanide thus inhibiting the growth of *S. roseus*.
- The investigation is best conducted in September when the leaves have been growing for a few months and the yeasts have had time to colonise and grow.
- Take leaves from the base of long shoots as these are the older leaves and have been on the tree since Spring. New young leaves from the tips of the shoots have fewer yeasts on them.
- After collecting the leaves keep them in a rigid container e.g. a plastic box, to prevent the leaves being crushed and the leaf yeasts from being rubbed off.
- After heavy rainfall or high wind, wait a few days to collect leaves as rain or wind may remove some of the leaf yeasts.
- Process the samples the same day that you collect them.
- Take care not to get petroleum jelly on the side of the leaf discs from which the spores are to be collected.
- Malt agar plates can be made up if they are not readily available.
- Variations on the investigation could be carried out as project work e.g. compare the leaf yeast populations from the upper and the lower leaf surfaces or from trees growing in different areas.