MiSAC activities 2

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

PYO ('pick your own') soil bacteria

The almost unimaginable diversity and versatility exhibited by micro-organisms is such that there is no one method which on its own is suitable for growing the entire microbial population of an environment. Neither is there a single method that is suitable for growing all the members of any one of the major groups of microbes, i.e. protozoa, algae, fungi, bacteria or viruses.

The problem is overcome by professional microbiologists by using selective methods and the enrichment culture technique to reveal the presence of specific microbes of importance in a particular circumstance, e.g. the pathogenic bacterium *Salmonella* in hospital diagnosis and food quality assurance.

The same principles can be applied to practical investigations that are suitable for use in the school laboratory.

In the following activities, specific members of the bacterial flora of soil are revealed by using (1) the selective effects of a physical factor (heat) and a chemical one (crystal violet); and (2) the enrichment culture technique for demonstrating cellulose degradation.

1. SELECTIVE METHODS FOR STUDYING SOIL BACTERIA

HEAT TREATMENT OF SOIL SUSPENSION*	CULTURE MEDIUM**		
	Nutrient agar (NA)[1]	NA + crystal violet[2]	Malt extract agar[3]
Unheated[a]			
60°C for 30 minutes[b]			[f]
80°C for 10 minutes[c]			[f]
100°C for 1 minutes[d]			[f]
121°C for 15 minutes[e]			

* 10% (w/v) soil in water.

** Inoculate by streak plate method but do not re-sterilise the loop during the procedure because of the small numbers of microbes present; secure the lid with adhesive tape; incubate at room temperature or 25°C for 2-7 days.

[1] A non-selective medium, pH value 7.4, allows development of only rapidly-growing bacteria; special methods are needed for those bacteria associated with soil fertility (e.g. in the nitrogen cycle) and decomposition (e.g. cellulose degradation as in activity 2).

[2] Crystal violet (10^{-5} final concentration) inhibits growth of Gram + but not Gram – bacteria.

[3] For comparing the effect on fungi and bacteria; pH value of the medium (5.4) inhibits bacteria but allows fungi to grow.

[a] Allows growth of a range of Gram + and Gram – bacteria.

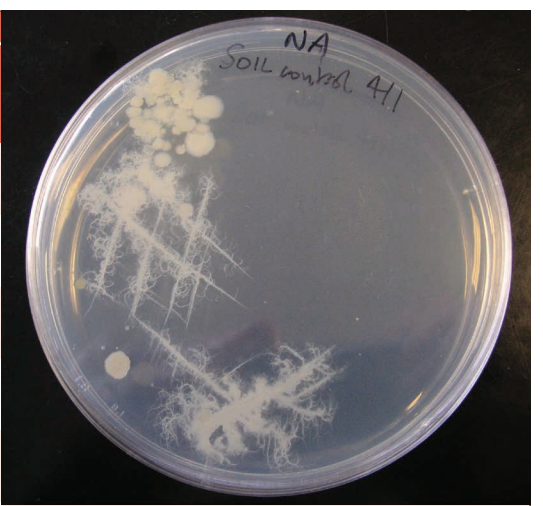
[b] Pasteurisation (compare definitions of 'Pasteurisation' and 'sterilisation').

[c] Those bacteria which form endospores survive and grow (all endospore-forming bacteria are Gram + but not all Gram + bacteria form endospores).

[d] Some endospore-forming bacteria survive and grow.

[e] Sterilisation conditions (autoclave or pressure cooker).

[f] Fungal spores are much less resistant than bacterial endospores to heat.



Colonies of bacteria on nutrient agar medium (above) and nutrient agar + crystal violet agar medium (below) inoculated from an unheated soil suspension by the streak plate method and incubated for 3 days at room temperature. Bacteria that produce 'curly' growth are common in soil and are not to be confused with fungi with which they may appear to be similar (see www.misac.org.uk/helpline_responses). Photographs of typical outcomes will be posted on www.misac.org.uk/practical_activities in due course.

2. THE CARBON CYCLE: THE ENRICHMENT CULTURE TECHNIQUE FOR STUDYING CELLULOSE DEGRADATION BY SOIL BACTERIA

Cellulose consists of long chains of glucose molecules which can be degraded by the cellulolytic enzymes (cellulases) produced by certain bacteria and fungi in the soil.

Cellulolytic microbes are present in soil in only small numbers but their presence can be demonstrated by using the enrichment culture technique. This technique provides special conditions which are not favourable for the growth of the rapidly-growing members of the microflora but enable others in the population to develop. In this activity, these conditions are achieved by using nutrient broth diluted to 10% (v/v) standard concentration.

The diluted medium does not contain sufficient amounts of readily-available sources of nutrients for the rapidly-growing bacteria to develop, thereby suppressing their growth and allowing other members of the microflora (in this case cellulolytic bacteria) to grow, i.e. become enriched. Filter paper provides the principal source of carbon and energy for the cellulolytic bacteria and becomes degraded in the process.

Requirements

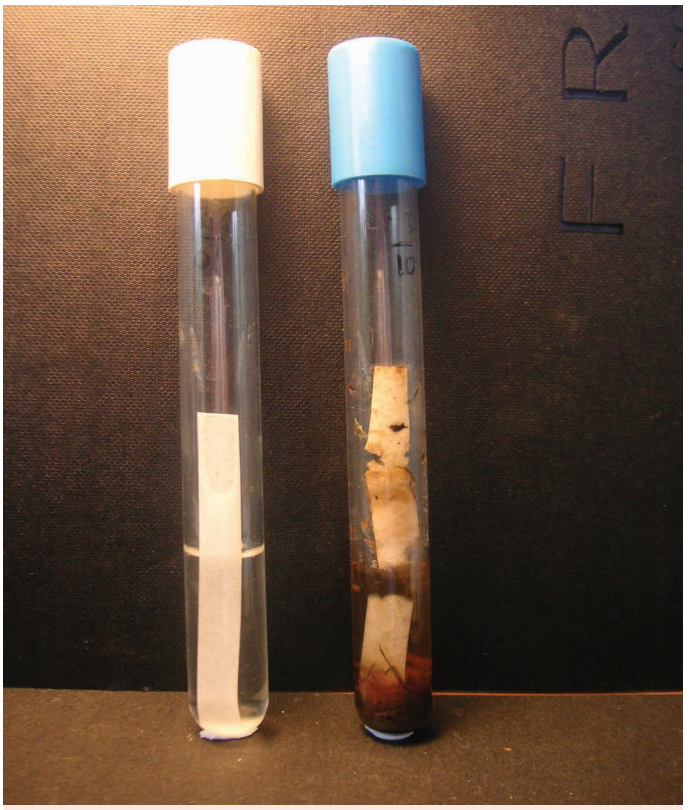
- Filter paper cut into strips, e.g. approximately 5 cm x 0.5 cm.
- Soil.
- Nutrient broth diluted to 10% (v/v) standard concentration.
- Test tubes closed with plastic caps or non-absorbent cotton wool plugs.

Procedure

1. Put the strips of filter paper into test tubes.
2. Prepare a soil suspension of approximately 10% (w/v) soil in 10% (v/v) nutrient broth.
3. Add approximately 5 cm^3 soil suspension to each test tube. Alternatively, dispense approximately 5 cm^3 of 10% (v/v) nutrient broth in test tubes and add 0.5 g soil to each.
4. Prepare a control tube of 10% (v/v) nutrient broth + filter paper strip without added soil.
5. Close the test tubes with either caps or plugs and incubate at room temperature or 25°C.
6. Examine the filter paper by eye for evidence of degradation at the culture medium-air interface. Check for weakening of the strip by firmly tapping the side of the tube. Marked effects are unlikely to be apparent after one week of incubation.
7. Continue incubation for another week, re-examining the tubes at convenient intervals.

Suggested extension work

- Compare different types of paper, e.g. writing (new and recycled), newspaper, magazine, cardboard.
- Investigate anaerobic degradation of cellulose by bacteria in fully-filled screw-cap bottles (e.g. 30 cm^3 capacity).
- Compare soil and compost (both 'home grown' compost and a commercial product).
- Compare nutrient broth at standard concentration and diluted to 1% (v/v) standard concentration.
- Investigate degradation of cellulose by fungi using malt extract broth; the pH value of this culture medium (5.4) allows fungi to grow but inhibits bacteria.



Cellulose degradation by soil bacteria in (above) aerobic and (below) anaerobic conditions; controls without soil are on the left.

HEALTH & SAFETY NOTES

1. Observe good microbiology laboratory practice (GMLP) throughout (for guidance see www.misac.org.uk/health and safety).
2. Students must examine cultures without opening plates, test tubes or bottles.
3. After use, all cultures must be disposed of safely by autoclaving.