Core practical 13: Isolate an individual species from a mixed culture of bacteria using streak plating

Objectives

- To successfully use streak plating techniques to isolate a single species of bacteria
- To understand the safety issues of microbiological work and how to apply good aseptic technique

Safety

- Wear eye protection.
- The microorganisms are a potential biological hazard.
- Students should not be allowed to use their streak plates (inoculated on day 1) when inoculating fresh streak plates on day 2. Instead, provide students with alternative plates inoculated by a suitably trained teacher/technician.
- Use aseptic techniques when transferring the bacteria to the Petri dishes.
- Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- If, on day 2, the streak plate from the mixed culture shows signs of contaminating growth, do not open the plate but destroy it by autoclaving.

Specification links

- Practical techniques 3, 9
- CPAC 1a, 2a, 2b, 3a, 3b

Procedure

Before this activity you should complete a risk assessment. This should detail the hazards, risks and precautionary measures that you will take during the procedure. A large part of this will record how you will avoid contamination of your cultures, yourself and the surrounding environment using aseptic techniques. The method below includes some such precautions but they are not exhaustive

Day 1

1. Wash your hands with soap and disinfect your bench area, leaving it to soak for 10 minutes before wiping it down. Light a Bunsen burner set to a safety flame on your working area.
2. With a marker pen, label the bottom of an agar plate with your name and the date.
3. Loosen the cap of the culture tube slightly.
4. Sterilise the inoculating loop by holding it in a hot Bunsen flame until the loop turns bright orange. Start near the handle end of the wire and work towards the loop.
5. Remove the loop from the flame and wait about 10 seconds for it to cool. Do not put it down. Using the little finger of the hand holding the loop, remove the top or stopper of the culture tube. Keeping the tube at an angle, pass the neck of the tube briefly through the flame, then dip the loop into the mixed culture broth. Replace the tube lid. Be careful as the neck of the tube may still be hot.
6. Take the loop and make streaks exactly as shown in fig A. Open the Petri dish lid, making sure you open it away from yourself and only open it slightly. Place the loop at area A (see fig A) and make at least three streaks from A to B. Then turn the Petri dish through a quarter turn and repeat from B to C, C to D and finally D to E. Each new set of streaks should pass through the tail end of the previous set. The final set of streaks should not touch the first set.
7. Flame the loop again before putting it down.

8. Place one small piece of tape on each side of the Petri dish lid to hold it down. Do not tape it all the way around. Invert the plate and incubate at a temperature no greater than 30 °C for 24 hours, or leave at room temperature for 48 hours.

Day 2

9. Keeping the lid on, observe your plate and sketch what has grown. There should be two types of colonies present on the agar; one species should be white and one should be yellow. If you have carried out the streak plating correctly there should be a number of completely separate colonies growing in the area of your final streaks. **Note: ensure the plates inoculated by students are labelled to ensure they are not accidentally opened.**
10. Obtain two nutrient agar plates and label them ‘white’ and ‘yellow’.
11. Turn on the Bunsen burner, flame an inoculating loop and allow to cool for 10 seconds.
12. Using the streak plates prepared by the teacher/technician choose a single white colony from the final set of streaks. Touch the loop against your chosen white colony to collect some bacteria and then streak onto the plate labelled 'white' in the pattern shown in fig A.
13. Flame the loop and cool.
14. Choose a yellow colony from the final set of streaks. Touch the loop on your chosen yellow colony and then streak onto the plate labelled 'yellow'.
15. Flame the loop again before putting it down.
16. Place one small piece of tape on each side of the lids to hold them down. Invert and incubate the plates at a temperature no greater than 30 °C for 24 hours, or leave at room temperature for 48 hours.

Day 3

17. Keeping the lids on, observe your plates. Sketch the appearance to show where bacterial growth occurred. Make notes on the colour and appearance of the bacterial colonies.
18. Clear away. Petri dishes with streak plates should be returned for sterilisation. Disinfect your bench area.

Notes on procedure

- Introduce students to the activity and ask them to produce a risk assessment before the practical. A demonstration of aseptic technique may be needed. It is also useful to demonstrate how agar plates are poured, although this will usually be done for the students. Teachers should be familiar with microbiology techniques.
- Careful planning will be needed to fit this practical activity around available lessons; each 'day' may not require a full lesson. Growth of plates can be slowed down if lessons are a couple of days apart by refrigerating Petri dishes. They should be contained and inverted. Remove them a couple of hours before the lesson to prevent condensation issues.
- All students should be given the opportunity to make at least one streak plate.
- A common problem with making streak plates is that students apply too much pressure and dig the loop into the agar. When making up nutrient agar, adding 0.5 g/100 ml of agar gives a thicker consistency which is easier to streak.
- *Micrococcus luteus* has yellow colonies while *Escherichia coli* and *Bacillus megaterium* have white colonies.

Answers to questions

1. Sets of streaks must cross over so that clumps of bacteria in the first streaks can gradually be spread out into subsequent streaks.
2. Temperatures above 30 °C are closer to human body temperature, so temperatures in this range would cause a risk of incubating human pathogens which could infect students.
3. The use of aseptic technique is vital in microbiology to ensure that there is no contamination of cultures by microorganisms from the environment and that people and the environment are not contaminated by the microorganisms being handled. Even cultures thought to be low risk should be treated with caution as bacteria may mutate to form pathogenic strains, our knowledge of the hazards may be incomplete or the culture may have become contaminated.

4. *Micrococcus luteus* forms yellow colonies and *Escherichia coli* and *Bacillus megaterium* form white colonies.
5. Ideas might include overall shape, shape of the colony margin, whether colonies are shiny or dull, and height of colony (flat or raised).

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Safety

- Wear eye protection.
- The microorganisms are a potential biological hazard.
- Do not open your inoculated plate! Instead use the alternative plate provided by your teacher.
- Use aseptic techniques when transferring the bacteria to the Petri dishes.
- Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- If, on day 2, the streak plate from the mixed culture shows signs of contaminating growth, do not open the plate. Your technician will destroy it by autoclaving.

Equipment

- bench spray of disinfectant
- paper towels or cloth
- Bunsen burner
- inoculating loop
- nutrient broth tube or bottle containing a mixed culture of bacteria
- 3 nutrient agar plates
- marker pen
- adhesive tape
- incubator

Diagram

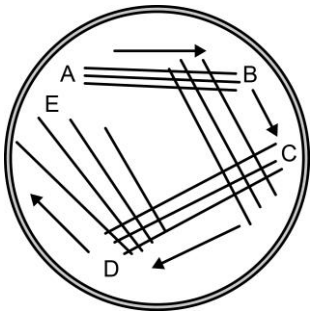

fig A Producing the streak plate.

Procedure

Before this activity you should complete a risk assessment. This should detail the hazards, risks and precautionary measures that you will take during the procedure. A large part of this will record how you will avoid contamination of your cultures, yourself and the surrounding environment using aseptic techniques. The method below includes some such precautions but they are not exhaustive.

Day 1

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3. Loosen the cap of the culture tube slightly.
4. Sterilise the inoculating loop by holding it in a hot Bunsen flame until the loop turns bright orange. Start near the handle end of the wire and work towards the loop.

5. Remove the loop from the flame and wait about 10 seconds for it to cool. Do not put it down. Using the little finger of the hand holding the loop, remove the top or stopper of the culture tube. Keeping the tube at an angle, pass the neck of the tube briefly through the flame, then dip the loop into the mixed culture broth. Replace the tube lid. Be careful as the neck of the tube may still be hot.
6. Take the loop and make streaks exactly as shown in fig A. Open the Petri dish lid, making sure you open it away from yourself and only open it slightly. Place the loop at area A (see fig A) and make at least three streaks from A to B. Then turn the Petri dish through a quarter turn and repeat from B to C, C to D and finally D to E. Each new set of streaks should pass through the tail end of the previous set. The final set of streaks should not touch the first set.
7. Flame the loop again before putting it down.
8. Place one small piece of tape on each side of the Petri dish lid to hold it down. Do not tape it all the way around. Invert the plate and incubate at a temperature no greater than 30 °C for 24 hours, or leave at room temperature for 48 hours.

Day 2

9. Keeping the lid on, observe your plate and sketch what has grown. There should be two types of colonies present on the agar; one species should be white and one should be yellow. If you have carried out the streak plating correctly there should be a number of completely separate colonies growing in the area of your final streaks.
10. Obtain two nutrient agar plates and label them ‘white’ and ‘yellow’.
11. Turn on the Bunsen burner, flame an inoculating loop and allow to cool for 10 seconds.
12. Using the alternative plate provided by your teacher, choose a single white colony from the final set of streaks. Touch the loop against your chosen white colony to collect some bacteria and then streak onto the plate labelled 'white' in the pattern shown in fig A.
13. Flame the loop and cool.
14. Choose a yellow colony from the final set of streaks. Touch the loop on your chosen yellow colony and then streak onto the plate labelled 'yellow'.
15. Flame the loop again before putting it down.
16. Place one small piece of tape on each side of the lids to hold them down. Invert and incubate the plates at a temperature no greater than 30 °C for 24 hours, or leave at room temperature for 48 hours.

Day 3

17. Keeping the lids on, observe your plates. Sketch the appearance to show where bacterial growth occurred. Make notes on the colour and appearance of the bacterial colonies.
18. Clear away. Petri dishes with streak plates should be returned for sterilisation. Disinfect your bench area.

Analysis of results

1. Write an evaluation of the success of your streak plating. For example, did you produce a good number of separate colonies that would have grown from one single bacterium? Were all the colonies in each separate plate the same colour and appearance or is there evidence of contamination?
2. Carry out some research to find out about the bacteria and identify which species are on each plate. Your teacher will tell you which two species were in the mixed culture.

Learning tip

- Most microorganisms require a good source of carbon and nitrogen as well as specific minerals. These nutrients can be supplied in a nutrient broth (liquid) or in a solid form. The solid medium used in your plates was nutrient agar (agar with some nutrients added), but many bacteria will not grow effectively on this simple medium. Agar is a jelly extracted from seaweed.

Questions

1. Why was it important that each set of streaks crossed over as seen in fig A?
2. Why should incubation be carried out below 30 °C?
3. Sterile (aseptic) technique is important in microbiology. Explain why this is the case even in this activity, where the bacterial species have been selected because they are harmless to humans.
4. Based on your research, which species was yellow and which was white?
5. Besides colour, what other features of colonies can be used to distinguish bacterial species?

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- Wear eye protection.
- The microorganisms are a potential biological hazard. Use aseptic techniques when transferring the bacteria to the Petri dishes. Disinfect the bench with 1% Virkon® or equivalent. Leave the disinfectant on the bench for about 10 minutes. This should be done before and after working.
- Students should not be allowed to use their streak plates (inoculated on day 1) when inoculating fresh streak plates on day 2. Instead, provide students with alternative plates inoculated by a suitably trained teacher/technician.
- Follow current best practice for handling microorganisms and sterilising or disposing of plates and used equipment. An autoclave or pressure cooker will be required for effective sterilisation of equipment and guidelines for safe use should be carefully followed.
- If, on day 2, the streak plate from the mixed culture shows signs of contaminating growth, do not open the plate but destroy it by autoclaving.

Equipment per student/group	Notes on equipment
bench disinfectant	1% Virkon® or equivalent. This should be left for about 10 minutes on the bench.
paper towels or cloth	To dry off the bench
Bunsen burner	One per group
inoculating loop	One per group
nutrient broth tube or bottle containing a mixed culture of bacteria	Each group needs only small quantities, sufficient to dip the inoculating loop into. This can be placed into a glass universal or McCartney bottle. If these are not available, sterile test tubes stoppered with cotton wool could be used. Label tubes as biohazard. Use *Escherichia coli* or *Bacillus megaterium* (which produce white colonies) and *Micrococcus luteus* (which produces yellow colonies). Inoculate each type of bacteria into separate nutrient broth cultures and grow for 24 hours before use. Mix together just before use.
nutrient agar plates	Each group needs one plate for day 1 and two plates for day 2. Once inoculated, label as a biohazard. A common problem with making streak plates is that students apply too much pressure and dig the loop into the agar. When making up nutrient agar, adding 0.5 g/100 ml of agar gives a thicker consistency which is easier to streak.

marker pen	
adhesive tape	
incubator	Set to no more than 30 °C.

Notes