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Version 3

Plate Streaking V.3

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Protocol status: Working

We use this protocol, and it's working

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Disclaimer

This protocol is used

Abstract

Overview and Goals

Streaking bacteria on agar plates allows scientists to obtain isolated colonies of bacteria. An isolated colony provides up to 10^8 bacteria cells that are genetically identical. This is why we call them clones or refer to them as colonies. The medium we chose to streak out bacteria must provide the nutrients required for prolific organismal growth, and not all bacteria require the same nutrients. There are various media types, and we will use Tryptic Soy Agar (TSA) plates to grow your bacteria, which may or may not support their growth.

Learning Objectives

After completing this lab, you will gain the following lab skills:

- Lab safety and proper personal protective equipment (**PPE**)
- Proper use of equipment: bacterial cell culture incubator
- **Bacterial plate streaking**
- Bacterial plate incubation

Image Attribution

Petri dish by [Jakob Vogel](#)

Purchased from The Noun Project



Guidelines

Safety

We are working with live bacterial organisms. Be careful not to contaminate your plate or spread the organism to surfaces. Wear gloves and eye protection for all steps.

Reducing Waste

To reduce unnecessary waste, we will tips for this activity. You can use the same tip to streak each plate. Then, switch tips for every isolate.

Materials

- Sharpie pen
- Wipes
- Sterile inoculation loops, Biolog Streakerz, or tips
- Pens and lab markers
- Bacterial culture source
- Inoculate plate with tryptic soy agar (TSA) medium

Safety warnings

 Follow all instructions provided by instructors. All tips, loops, and gloves will go into biohazard bins.

Ethics statement

This protocol does not involve animals.

Before start

Preparation

Learn how to perform bacterial plate streaking to obtain isolated colonies.

1. Watch **Streaking for Isolation** (3:38 min)

<https://www.youtube.com/embed/Fz4qp1ulvT4>

1. Read this article to learn about ways to streak a plate to obtain isolated colonies: **Streak Plate Method- Principle, Types, Methods, Uses** (15 min)
 - Practice with a pen and paper the "Quadrant Streaking Method."

Labeling a Plate

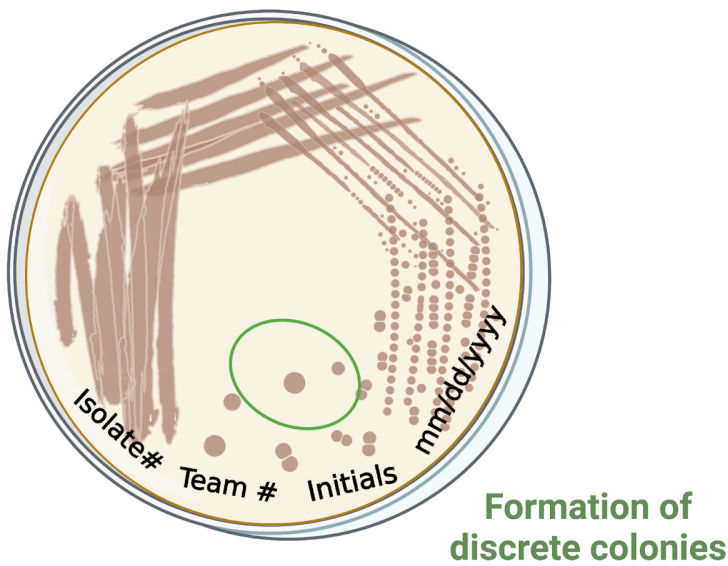


Figure 1. Illustration of plate with bacterial colonies. The plate is labeled with isolate #, Team #, Initials, and date (mm/dd/yyyy). Illustration created with BioRender.com.

Plate Streaking Method

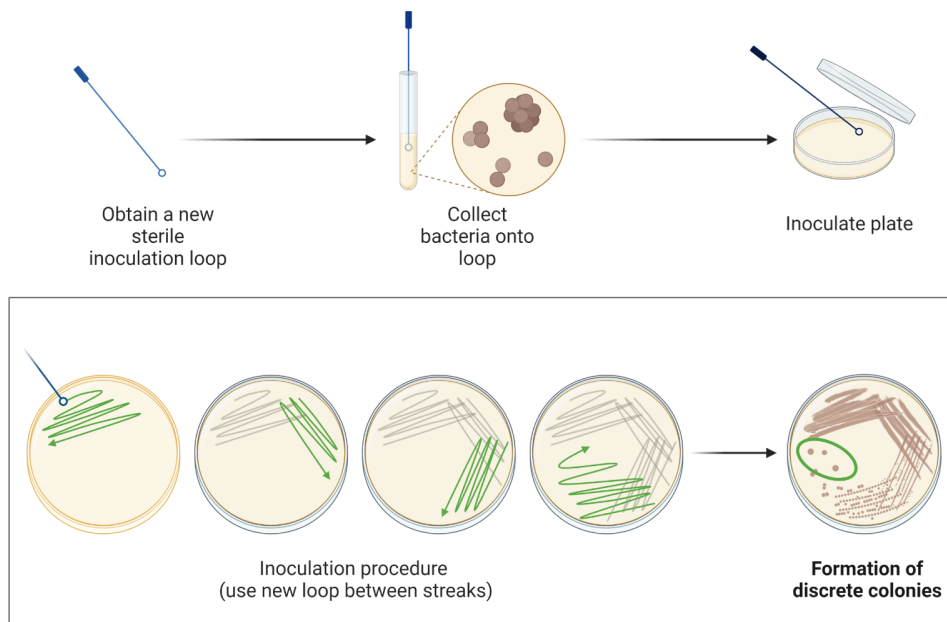


Figure 2. Diagram with steps for streaking a plate.

- 1) Obtain a new sterile inoculation loop
 - 2) Collect bacteria onto loop
 - 3) Inoculate plate
 - 4) Streak plate using new loop between streaks
 - 5) Observe formation of discrete colonies
- Illustration created with BioRender.com.



Procedure for plate streaking

1d 0h 32m 30s

- 1 Obtain a fresh Petri dish containing tryptic soy agar (TSA) medium. 5m
- 2 Label the bottom of the plate (the side containing TSA medium) keeping to the circumference (write on the edge, not in the center) with your bacterial isolate#, your Team#, your initials and today's date. See the diagram below for a visual example. If the surface of the plate is wet, dry it off using a chemical wipe. 5m
- 3 Obtain a **sterile loop, tip, or Streakerz**. 3m
- 4 Inspect the plate with the bacterial isolate you will characterize. 2m
- 5 Using the sterile plastic loop, touch the loop to a single colony on your bacterial source plate. 30s
- 6 Streak the bacteria on the loop onto your labeled inoculate plate following the inoculation procedure illustrated above. 15m
- 7 Put your lid back on your plate, turn it upside down (lid facing the benchtop) and bring it to the incubation chamber. 2m
- 8 Incubate your bacterial plate overnight with the goal of obtaining single isolated colonies. This would be the result 1d



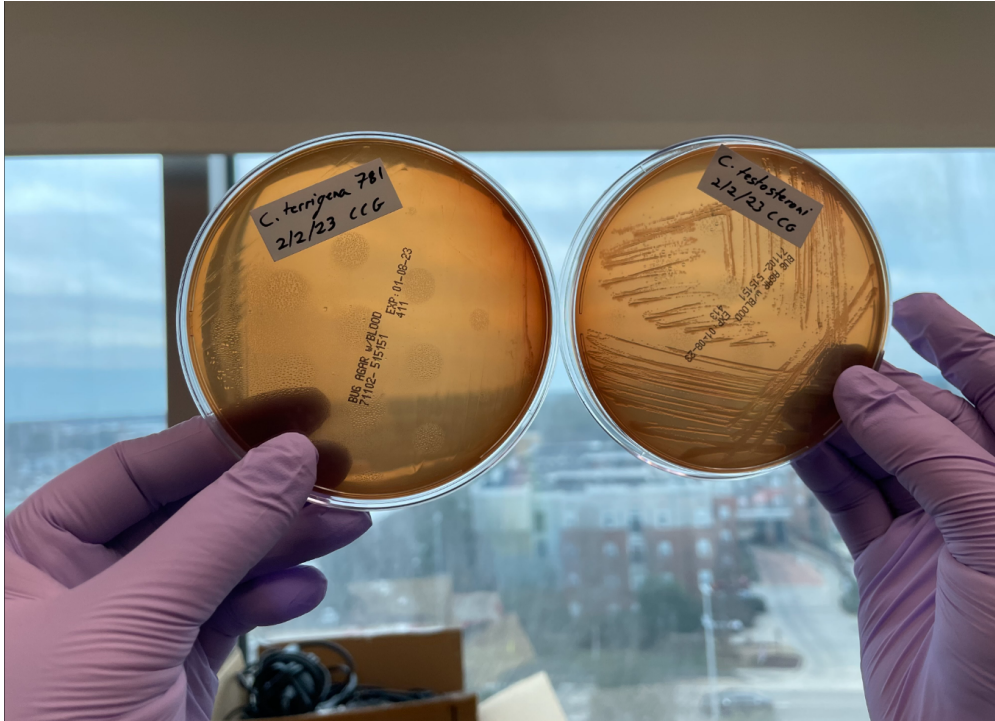


Photo of two BUG agar plates (Biolog) with *Comamonas terrigena* (left) and *Comamonas testosteroni* (right) held by gloved hands. The plate on the right shows growth in all four streaks. Photo taken by BIT 295 Staff.

Note

Critical Thinking Questions

1. When plating bacteria, why do you think the last of the successive streaking turns will result in individual colonies as opposed to thick lines of bacteria seen at the beginning?
2. Why do you think researchers put plates into an incubator upside down (lids on the bottom)?

Acknowledgements

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