Effect of Temperature on Bacteria Growth and The Use of Selective and differential Media

Purpose

Using "Borderless Lab 365" platform to understand the effect of temperature on bacteria growth; and identify bacteria species using selective and differential media through observing the colonies' appearance and the color changes of the media.

Background

- Bacteria grow by dividing one bacterium into two daughter cells in the process called binary fission. All bacteria have their own **optimal growth temperature**. There are 5 classifications of bacteria based on their optimal growth temperature, including psychrophiles, psychrotrophs, mesophiles, thermophiles, and hyperthermophiles. Most of the human pathogens are mesophiles which grow best between 25-40°C. *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are examples of mesophiles that will be used in this experiment.

- **Selective Media** contains chemical substances which can suppress the growth of unwanted microbes or encourage the growth of desired microbes. **Differential media** can be used to distinguish bacteria based on their morphology and biochemically related groups of organisms, causing characteristic change in appearance of bacterial growth and/or medium surrounding the colonies which permits differentiation. Mannitol Salt Agar and MacConkey Agar are used in this experiment.

- **Mannitol salt agar**: It contains a high salt concentration, 7.5% NaCl, which inhibits the growth of most bacteria except *Staphylococci*. It also performs a differential function as it contains the carbohydrate 'mannitol', which some *Staphylococci* are capable of fermenting and we can use phenol red (a pH indicator) for detecting acid produced by mannitol-fermenting *Staphylococci*. Mannitol-fermenting *Staphylococci* exhibit a yellow zone surrounding their growth, while *Staphylococci* that do not ferment mannitol will not produce color changes.

- **MacConkey agar**: It contains crystal violet which prevents the growth of Gram-positive organisms so we can isolate Gram-negative bacteria by inoculating them in MacConkey agar. It contains the carbohydrate lactose, bile salts and the pH indicator neutral red, permitting differentiation of enteric bacteria on the basis of their ability to ferment lactose. On this basis, enteric bacteria are separated into two groups. Bacteria that can ferment lactose cause colonies growth to appear in pink or red.

Materials

Mannitol Salt Agar & MacConkey Agar Powder
8 Petri Dishes (60mm Diameter)
Escherichia coli (*E. coli*)
Staphylococcus aureus (*S. aureus*)
Electronic balance
Weighting tray
Spatula
250mL measuring cylinder
Distilled water
2 250mL media bottles
Water bath
Bunsen Burner
Inoculation loop
2 incubator boxes at 20°C and 37°C

Preparation

1. Clean the bench surface using 70% ethanol.

2. Prepare 4 Mannitol Salt Agar plates and 4 MacConkey agar plates.

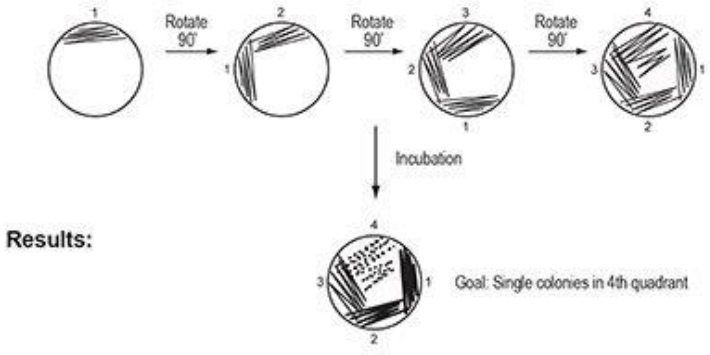
a. Dissolve 27.75g Mannitol Salt Agar powder in 250ml of distilled water in 250ml media bottle.
b. Dissolve 12.875g MacConkey Agar Powder in 250ml of distilled water.
c. Stir and label the flask with autoclave tape.
d. Sterilize the Mannitol Salt Agar solution and the MacConkey Agar solution by autoclaving at 121°C for 20 minutes.
e. Prepare water bath and set the temperature to 50°C.
f. After sterilization, cool down the agar solution by putting the glass bottle into the 50°C water bath for 5 minutes.
g. Turn on the Bunsen burner.
h. Label the petri dish with the name of agar, bacteria, and temperature.
i. Pour approximately 15mL of agar solution into each Petri dish. Make sure that the environment is sterile (bench is cleaned using 70% ethanol and work is being done near the Bunsen burner.)
j. Allow the solution to be cooled down and solidified at room temperature for about 20 minutes and flip to avoid condensation on the agar.
k. Dry the Petri dishes by putting in a 37°C incubator for 2-3hours, or room temperature for 2-3 days. Store the Petri dishes in fridge at 4°C if the plates are not used immediately.

3. Transfer bacteria on all 8 petri dishes.

a. Clean the Bench with 70% ethanol.

b. Turn on the Bunsen burner.
c. Take out the prepared plates and check the plates. Repeat step 2 if the plates are contaminated.
d. Flame the inoculation loop using the Bunsen burner until it is reddish in color to sterilize it.
e. Cool the inoculation loop down for a few seconds.
f. Select one colony of *E. coli* and *S. aureus* from the bacterial plates culture and transfer them to the agar plates using the inoculation loop. (Each type of bacteria transfer to 2 Mannitol Salt Agar plates and 2 MacConkey agar plates)
g. Spread the bacteria by streaking.

A

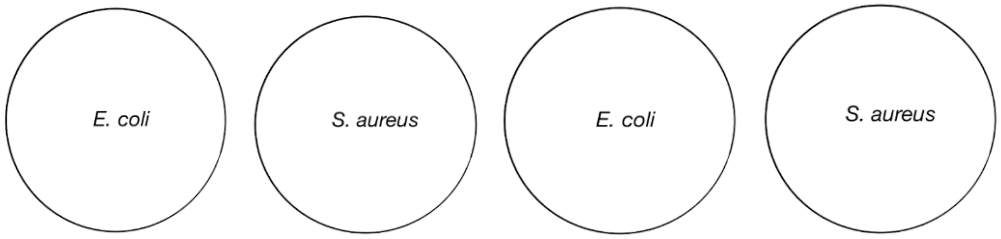


B

h. Put the petri dishes into the incubator of 37°C and 20°C for 24 hours.

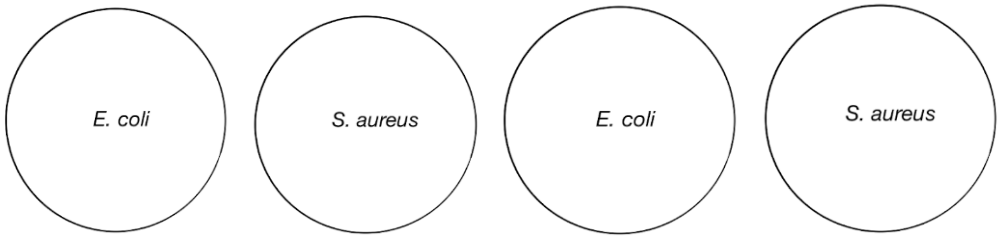
Box 1 (37°C)

Petri Dish 1,2: Mannitol Salt Agar Plate Petri Dish 3,4: MacConkey Agar Plate



Box 2 (20°C)

Petri Dish 1,2: Mannitol Salt Agar Plate Petri Dish 3,4: MacConkey Agar Plate



****All the steps have been done in The Hong Kong Polytechnic University**

Procedure

1 Log in the experiment module "Bacterial Growth" on the Borderless Lab 365 platform. https://stem-ap.polyu.edu.hk/remotelab/

2 Observe the sample every hour.

3 Download all photos.

4 Press "LOGOUT" on the left when you complete the experiment

Results

Observe the results and fill in the table.

Box 1 (37°C)

	Mannitol Salt Agar Plate		**MacConkey Agar Plate**	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***
Presence of colonies	Yes / No			
Number of bacteria	Many/Few/NA			
Color of colonies				
Color of agar				

Box 2 (20°C)

	Mannitol Salt Agar Plate		**MacConkey Agar Plate**	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***
Presence of colonies				
Number of bacteria				
Color of colonies				
Color of agar				

Discussion

1 What is the optimal temperature for the growth of *E. coli and S. aureus*?

2 What are the differences between Gram-positive and Gram-negative bacteria?

3 Is *E. coli* Gram-positive or Gram-negative bacteria?

4 Is *S. aureus* Gram-positive or Gram-negative bacteria?

5 Does *E. coli* ferment lactose or mannitol?

6 Does *S. aureus* ferment lactose or mannitol*?*

7 Give 2 other examples of Gram-positive bacteria.

8 Give 2 other examples of Gram-negative bacteria.