

# BACTERIA GROWTH CHARACTERISTICS

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## OBJECTIVE/RATIONALE

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Recognizing colony growth characteristics and biochemical reactions to various media is important in identifying bacteria. The student will plate known bacteria and observe for colony characteristics and biochemical reactions.

TEKS 121.14 (c) 1A, 1B, 4B, 4C, 4D

TAKS ELA 1, 4  
Mathematics 8  
Science 1, 2, 3

National Science Education Standards A9-12;C9-12

National Health Care Skills Standards .01, .04, .05, .06, .07, .08

National Curriculum Standards for School Mathematics S1; S3

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## KEY POINTS

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- I. Introduction
  - a. Bacteria have different characteristic morphology of their colonies. These colonies will take on additional characteristics that aid in differentiation depending on the media/agar used for growth.
- II. Colony characteristics:
  - a. The Colony Surface
    - 1. Smooth
    - 2. Rough
    - 3. Wrinkled
    - 4. Glistening
  - b. Colony Color
    - 1. Describe the color of the colony
      - a. Soluble: Does the color diffuse into the media/agar?
      - b. Insoluble: Does the color stay within the colony?
      - c. Color: white-white, white-gray, light purple, yellow-brown, etc.
  - c. Consistency:
    - 1. Take an inoculating loop and poke at the colony. Describe it by one of the following:
      - a. Mucoid: resembling mucous
      - b. Dry: crusty, brittle
      - c. Soft: butter-like consistency
  - d. Visual characteristics
    - 1. Translucent: See-through
    - 2. Opaque: No light shines through
    - 3. Iridescent: Rainbow colors in reflected light
    - 4. Dull: No shine to the colony
    - 5. Shiny: Reflects light
  - e. Shape of the colony (See handout for pictures)

1. Circular
  2. Irregular
  3. Spindle
  4. Filamentous
  5. Spreading
- f. Height of colony (See handout for pictures)
1. Raised
  2. Flat
  3. Convex
  4. Heaped
  5. Sunken

### III. Media and Biochemical Reactions

- A. MSA-Mannitol Salt Agar: a selective medium for the isolation of Staphylococci species. It contains a 7.5% NaCl that inhibits the growth of most gram-negative and gram-positive microorganisms. MSA contains beef extract as a nutrient and phenol red indicator. When Staphylococcus aureus is grown, it ferments the sugar mannitol, producing yellow colonies. Those Staphylococcus species that do not ferment mannitol, remain as small red colonies and the plate remains pink to red.
- B. MAC-MacConkey: a selective, differentiating medium for the isolation of gram-negative bacilli and differentiation of lactose fermenters from nonlactose fermenters. It contains bile salt and crystal violet that inhibit the growth of gram-positive bacteria. If the microorganism can ferment lactose, the colonies appear pink to red in color and if it cannot ferment lactose, the colonies appear clear. The medium also contains lactose and neutral red indicator.
- C. BLD-Sheep blood agar: an enriched, non-selective medium. It is trypticase soy agar with 5% sheep red blood cells added to it. Gram-positive and gram-negative bacteria can grow on this medium. Hemolytic patterns of certain bacteria may be seen on the plate:
1. Alpha hemolysis: incomplete hemolysis; greening of the medium
  2. Beta hemolysis: total clearing of the medium
  3. Gamma hemolysis: nonhemolytic; no change in the color of the medium.
- D. EMB-Eosin methylene blue agar: a selective, differential medium for the isolation of gram-negative bacilli and differentiation of lactose fermenters from nonlactose fermenters. The medium contains lactose, eosin dye, and methylene blue dye. The dyes are toxic to gram-positive bacteria. When bacteria ferment the lactose, the pH decreases in the medium and the medium changes to a purple color. Gram-negative bacteria that ferment lactose appear with a green metallic sheen. Those that cannot ferment lactose appear clear on the medium.
- E. CNA-Colistin-nalidixic acid agar: for isolation of gram-positive bacteria. The medium contains sheep blood agar and the antibiotics colistin and nalidixic acid added. Colistin disrupts the cell membrane of gram-negative

microorganisms and the nalidixic acid blocks DNA replication in the gram-negative bacteria.

- F. PEA-Phenylethyl alcohol agar: selective medium that inhibits the swarming of Proteus species. Gram-positive facultative anaerobes such as Staphylococcus, Streptococcus, and Corynebacterium grow on this medium.
- G. TSA-Trypticase soy agar: a general purpose medium for isolation of fastidious microorganisms. The medium contains nutrients for both gram-negative and gram-positive bacteria.
- H. CHOC-Chocolate agar: an enriched non-selective medium to isolate fastidious microorganisms. Neisseria species and Haemophilus species grow well on this medium. This medium is heated to destroy red blood cells and release NAD to aid in growth of bacteria. It also has IsoVitaleX, which contains dextrose, cysteine, vitamin B12, thiamine, and ferric nitrate.
- I. TSB-Trypticase Soy Broth: a general purpose medium for all types of microorganisms. This medium is in a liquid form that is great to use for growth of stock cultures.
- J. UREA BROTH: for identification of urease producers, such as Proteus, Morganella, Klebsiella pneumoniae, and some Enterobacter species. The enzyme, urease, splits urea into alkaline end products. This results in an increased pH of the medium and a color change in the indicator to pink-red.
- K. GN BROTH: an enrichment broth used to inhibit gram-positive microorganisms. It contains bile salt that is toxic to gram-positive bacteria and is inhibitory to normal flora coliforms of the digestive system.

#### GLOSSARY OF TERMS

- 1. Differential media: primary isolation media that is selective for a specific group of bacteria.
  - 2. Enrichment media or broth: enhances the growth of groups of bacteria.
  - 3. Fastidious: bacteria that have complex nutritional requirements.
  - 4. Hemolysis: destruction of red blood cells.
  - 5. Coliforms: gram-negative bacilli that ferment lactose, i.e. Escherichia coli.
- IV. Important Point to Remember
- a. Examine the colonies with light sources above and below the plate.
  - b. Color description should be extremely specific.
  - c. Examine well isolated colonies.
  - d. Check for changes in appearance of the media/agar surrounding the colony.
  - e. Note any unusual characteristics, i. e. a fried egg appearance of the colony.
  - f. Enrichment media may cause some colonies to grow larger than others and some dyes may cause the colonies to be brightly colored.

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### ACTIVITIES

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- I. Complete the Bacteria Growth Characteristics Laboratory Investigation.

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## **MATERIALS/RESOURCES**

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### Bacterial Morphology Handout

Bacteria to be used:

Bacillus subtilis  
Escherichia coli  
Proteus mirabilis  
Pseudomonas aeruginosa  
Enterococcus faecalis  
Micrococcus luteus  
Staphylococcus aureus  
Staphylococcus epidermidis  
Bacillus cereus  
Serratia marcescens

Media to be used:

MSA  
MAC  
BLD  
EMB  
CNA  
PEA  
TSA  
CHOC  
TSB  
UREA BROTH  
GN BROTH

Inoculating loop

Bunsen burner

Gloves

Goggles

Incubator 37C

Lab coats

Disinfecting Solution

Paper towels

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## **ASSESSMENT**

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### Laboratory Investigation Rubric

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## **ACCOMMODATIONS**

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For reinforcement, the student will design a chart of colony characteristics and biochemical reactions.

For enrichment, the student will identify an unknown bacteria using colony characteristics and biochemical reactions.

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## **REFLECTIONS**

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# BACTERIALS MORPHOLOGY

## COLONY SHAPES:

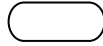
**Circular**



**Irregular**



**Spindle**



**Filamentous**



**Spreading**



## COLONY HEIGHT:

**Raised**



**Flat**



**Convex**



**Heaped**



**Sunken**



# **BACTERIA GROWTH CHARACTERISTICS**

## **LABORATORY INVESTIGATION**

### **PURPOSE:**

In this laboratory investigation, the student will plate known bacteria and observe for colony characteristics and biochemical reactions.

### **BACKGROUND INFORMATION:**

### **MATERIALS:**

Bacteria listed below  
Media Listed below  
Inoculating loop  
Bunsen burner  
Gloves  
Goggles  
Incubator 37C  
Lab coats  
Disinfecting Solution  
Paper towels

### **PROCEDURE:**

Plate the following bacteria on the list of media printed below, using the plating technique you have learned. Be sure to use standard precautions and treat every specimen as a potential pathogen.

*Bacillus subtilis*

*Escherichia coli*

*Proteus mirabilis*

*Pseudomonas aeruginosa*

*Enterococcus faecalis*

*Micrococcus luteus*

*Staphylococcus aureus*

*Staphylococcus epidermidis*

*Bacillus cereus*

*Serratia marcescens*

Media to be used:

MSA

MAC

BLD

EMB

CNA

PEA

TSA

CHOC

TSB

UREA BROTH

GN BROTH

**DATA:** Record the growth, colony characteristics, and biochemical reactions (if applicable) for each of the following bacteria:

**Bacillus subtilis:**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Escherichia coli**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Proteus mirabilis**

MSA:	MAC:
BLD:	EMB:
CNA:	PEA:
TSA:	CHOC:
TSB:	UREA BROTH:
GN BROTH:	

**Pseudomonas aeruginosa**

MSA:	MAC:
BLD:	EMB:
CNA:	PEA:
TSA:	CHOC:
TSB:	UREA BROTH:
GN BROTH:	

**Enterococcus faecalis**

MSA:	MAC:
BLD:	EMB:
CNA:	PEA:
TSA:	CHOC:
TSB:	UREA BROTH:
GN BROTH:	

**Micrococcus luteus**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Staphylococcus aureus**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Staphylococcus epidermidis**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Bacillus cereus**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**Serratia marcescens**

MSA:

MAC:

BLD:

EMB:

CNA:

PEA:

TSA:

CHOC:

TSB:

UREA BROTH:

GN BROTH:

**CONCLUSION:**

Prepare a cross reference list of each bacteria that grew on each plate.