

Bacteria Growth Characteristics

Course

Medical
Microbiology

Unit VI

Concepts of
Medical
Microbiology

Essential Question

How can we
macroscopically
identify
bacteria?

TEKS

130.207(c)1A,
2FGH, 4BCEF,
5E

Prior Student Learning

Growth of
Microorganisms

Estimated time

Introduction of
key points and
lab review: 30-
50 minutes

Lab procedure:

30-50 minutes
(24hr. incubation
needed before
observation)

Rationale

Recognizing colony growth characteristics and biochemical reactions to various media is important in identifying bacteria.

Objectives

Upon completion of this lesson, the student will be able to:

- Plate known bacteria and observe for colony characteristics and biochemical reactions

Engage

Present handout of the photos below (or a similar collection) and ask students to identify the different breeds. Then explain:

Much in the same way we can recognize different breeds of dogs by appearance, microbiologists can also recognize different species of bacteria by macroscopic (naked eye – colony appearance) and microscopic appearance (usually gram stain under a microscope).



All photos courtesy of www.CDC.gov

Key Points

- 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 (or texture)
 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; which word most closely describes it?
 - a. mucoid: resembling mucous
 - b. dry: crusty, brittle
 - c. soft: butter-like consistency

D. Visual characteristics – appearance in light

- 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. Scent of the colony (that lovely after a fresh rain smell is new bacterial growth)

- 1. No Odor
- 2. Musty
- 3. Sour
- 4. Grapes or corn tortillas (very typical of *Pseudomonas*)

G. 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 non-lactose 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: non-hemolytic; 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 non-lactose 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-Phenyl ethyl 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 USED IN SECTION III

- 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 Points 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.

Activity

- I. Complete the Bacteria Growth Characteristics Laboratory Investigation.
- II. Optional activity: if you are unable to have media with bacteria in your

classroom gather photos or use a photo atlas and have students analyze the photos for the same listed qualities. (Consistency will need to be given)

Assessment

Laboratory Investigation Rubric

Materials

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

*Minimum Suggested Set

Inoculating loop (metal reusable or plastic disposable)

Bunsen burner (not needed if using plastic loops)

Gloves

Goggles

Incubator 37°C

Lab coats

Disinfecting Solution

Paper towels

Accommodations for Learning Differences

For reinforcement, the student will graph colony characteristics from several species to find similarities.

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

National and State Education Standards

National Health Science Cluster Standards

HLC01.01 Academic Foundations – Health care workers will know the academic subject matter required (in addition to state high school requirements for high school graduation) for proficiency within their area.

They will use this knowledge as needed in their role. – Use a knowledge of diseases and disorders. – Analyze methods to control the spread of

pathogenic microorganisms.

TEKS

130.207(c)1(A) Demonstrate safe practices during laboratory and field investigations

2(F) Collect and organize qualitative and quantitative data and make measurements with accuracy and precision using tools such as calculators, spreadsheets, software, data collection probes, computers, standard laboratory glassware, microscopes, various prepared slides, stereoscopes, metric rulers, electronic balances, hand lenses, Celsius thermometers, hot plates, lab notebooks or journals, timing devices, Petri dishes, lab incubators, dissection equipment, meter sticks, and models, diagrams, or samples of biological specimens or structures.

2(G) Analyze, evaluate, make inferences and predict trends from data

2(H) Communicate valid conclusions supported by the data through methods such as lab reports, labeled drawings, graphic organizers, journals, summaries, oral reports, and technology based reports.

4(B) Identify chemical processes of microorganisms

4(C) Recognize the factors required for microbial reproduction and growth.

4(E) Describe the morphology and characteristics of microorganisms using a variety of microbiological techniques.

4(F) Discuss the results of laboratory procedures that are used to identify microorganisms

5(E) Evaluate the effects of anti-microbial agents

Texas College and Career Readiness Standards

Mathematics Standards – VI. Statistical Reasoning –

B. 2. Select and apply appropriate visual representations of data. 4. Describe patterns and departure from patterns in a set of data.

C2. Analyze data sets using graphs and summary statistics.

Science Standards – I. Nature of Science –

C. 2. Understand and apply safe procedures in the laboratory field, including chemical, electrical, and fire safety and safe handling of live or preserved organisms. 3. Demonstrate skill in safe use of a wide variety of apparatuses, equipment, techniques, and procedures.

Cross-Disciplinary Standards – II. Foundational Skills – D. 1. Identify patterns or departures from patterns among data. 3. Present analyzed data and communicate findings in a variety of formats.

BACTERIALS MORPHOLOGY

Colony Characteristics

Colony Shapes:

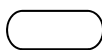
Circular



Irregular



Spindle



Filamentous



Spreading



Colony Height:

Raised



Flat



Convex



Heaped



Sunken



Colony Surface:

Smooth



Rough



Wrinkled

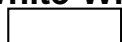


Glistening

Reflective and shiny

Colony Color:

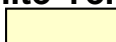
White-White



White-Grey



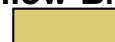
White-Yellow



Light Purple



Yellow-Brown



Soluble – does the color diffuse into the media/agar?

Insoluble – does the color stay with the colony?

Colony Consistency: (check by touching the colony with an inoculating loop)

Mucoid – sticky like mucous

Dry – crusty and brittle

Soft – butter-like consistency

Colony Visual Characteristics:

Translucent – see through

Opaque – no light shines through

Iridescent – rainbow colors in reflected light

Dull – no shine to the colony

Shiny – reflects light

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 (metal or plastic)
Bunsen burner (only needed with metal loops)
Gloves
Goggles
Incubator 37°C
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.

Bacteria to be Used :

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

*Minimum Set

Media to be used:

MSA (Mannitol Salt Agar)
MAC* (MacConkey Agar)
BLD* (Sheep's Blood Agar)
EMB (Eosin Methylene Blue Agar)
CNA (Colistin-Nalidixic Acid Agar)
PEA (Phenyl ethyl Alcohol Agar)
TSA (Trypticase Soy Agar)
CHOC* (Chocolate Agar – hemolyzed RBCs)
TSB* (Trypticase Soy Broth)
UREA BROTH
GN BROTH (Gram Negative)

DATA: Record the species in the bacteria box and note growth, colony characteristics and biochemical reactions (if applicable) for each growth media used. Repeat for each species.

Bacteria:	Color	Shape	Height	Surface	Consistency	Visual Char.	Biochemical Reaction	No Growth	Scent
MSA									
MAC									
BLD									
EMB									
CAN									
PEA									
TSA									
CHOC									
TSB									
Urea B									
GN B									
Bacteria:	Color	Shape	Height	Surface	Consistency	Visual Char.	Biochemical Reaction	No Growth	Scent
MSA									
MAC									
BLD									
EMB									
CAN									
PEA									
TSA									
CHOC									
TSB									
Urea B									
GN B									

Laboratory Investigation Rubric

Student: _____ Date: _____

Scoring Criteria	4 Excellent	3 Good	2 Needs Some Improvement	1 Needs Much Improvement
Problem is appropriately identified.				
Problem is precise, clear, and relevant.				
Association between the problem and the predicted results is direct and relevant.				
All variables are clearly operationalized.				
Demonstrates comprehension of the use of scientific concepts and vocabulary.				
All significant data is measured.				
Data is recorded effectively and efficiently.				
Data table is well designed to the task requirements.				
All graphs are appropriate.				
All data accurately plotted.				
Graph visually compelling; highlights conclusions of the study.				
Conclusion relates directly to the hypothesis.				
Conclusion has relevancy in resolution of the original problem.				
Conclusion relates the study to general interest.				

Engage Activity



All photos courtesy of www.CDC.gov