EXPERIMENT 7

Human Bacteriology

INTRODUCTION

Besides the natural environment, bacteria are a major component of the human microbiome. Most parts of the human body contain bacteria and is not restricted to skin, throat, saliva, and intestines. Bacteria found in the body are generally considered “normal flora” and may or may not be beneficial to the human host. When the normal flora of the body does not cause harm to the host they are often termed **commensals**. At times, given the right opportunity (open sore, wound) these bacteria can take advantage and become pathogenic.

Normal flora on and within the body is extremely important to a healthy human. Normal flora prevents and inhibits the growth of other microbes by being already established (often from natural birth) and utilizing the available nutrients. Additionally, normal flora is responsible for producing certain enzymes that inhibit the growth of incoming bacteria. Gut bacteria are an excellent example of the human dependence on bacteria microbiome. Human gut bacteria are responsible for the break down of compounds that are incapable of breakdown by humans including fiber. These bacteria also make important B (especially B12) and K vitamins available for our development where we would otherwise be unable to absorb.

Different parts of the human body will host different bacteria. For example, the bacteria found within the body will very often be different from the bacteria found on the skin. The body contains different microenvironments where bacteria have evolved to inhabit. The skin often hosts bacteria capable of salty environments because of our sweat, whereas anaerobic bacteria that require oxygen-deprived environments inhabit the throat and GI tract.

In this experiment, you will be using both selective and differential media. **Selective media** involves the use of any physiological component (or condition) that allows for the growth of particular physiological types of bacteria. Such physiological component can include the use of high salt or sugar concentrations, or changing the pH of the medium. **Differential media**

involves the use of chemical ingredients that react with certain microbes to differentiate, or discern, between different bacterial types based on colony morphology or reactions with the medium. Differential chemical compounds often involve dyes that react with certain bacteria.

Additionally, other media types are often used in the lab, and in this experiment. In microbiology, Complex and Chemically Defined Media are often used to isolate varying microorganisms. **Complex media** is one where the exact chemical composition is unknown and is used to grow a wide range of different types of microbes. A common complex media that is used in the lab to grow random assemblages of bacteria is Nutrient Agar. **Chemically defined media** is one where the media composition is known and exact from batch to batch.

In today's experiment, we will sample bacteria from varying parts of the body and isolate them on three different media: Mannitol Salt Agar, Nutrient Agar, and Blood Agar. Nutrient agar is a complex media and is neither selective nor is it differential, instead it will grow a wide array of bacteria. Mannitol Salt Agar (MSA) is both selective and differential. MSA is selective in that it contains 7.5% NaCl where only halophiles or halotolerant bacteria are able to grow. MSA also contains the sugar Mannitol and the dye phenol red. Phenol red is a dye that is red in neutral conditions and turns yellow in acidic conditions. When a bacterium is able to grow on the MSA agar, it will use the Mannitol sugar and produce acids, these acids will in turn lower the pH of the agar and change the Phenol red into a yellow color (Figure 1).

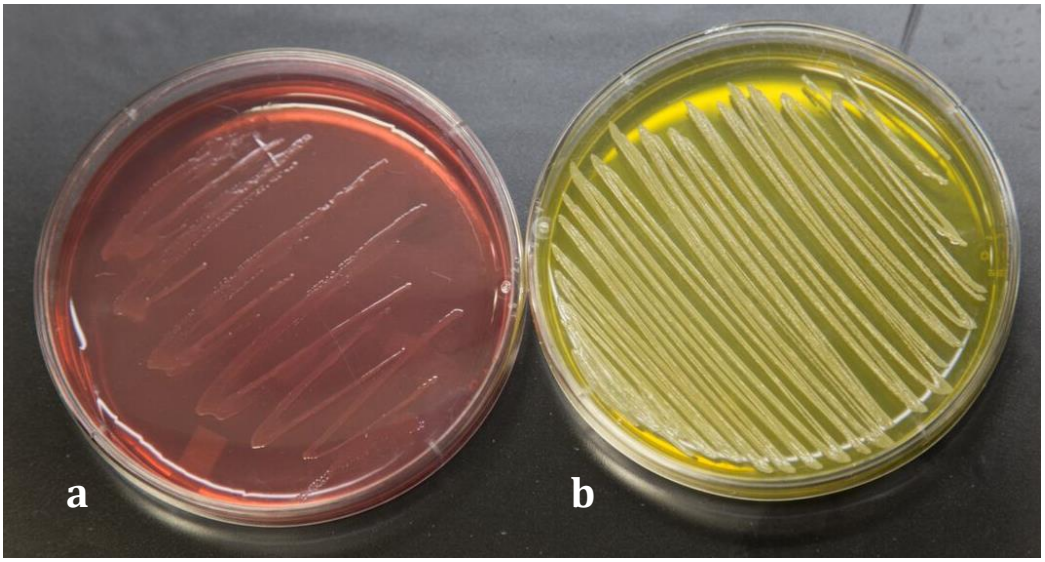

Figure 1. Two halophilic/halotolerant bacteria growing on MSA plates, with only one able to produce acids from Mannitol sugar (b).

Common bacteria that you will encounter on the skin of humans include the aerobic gram-positives *Staphylococcus epidermis*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Propionibacterium acnes*.

The other plate used in this experiment in order to isolate human derived bacteria is the Blood Agar plate (BA). BA plates are differential media that uses 5% sheeps blood in order to show different reactions of bacteria based on pathogenicity. Many severe human infections can be caused by bacteria in the normal flora. Bacteria can produce hemolysis, or hemolytic reactions, that act on red blood cells. On BA two types of hemolysis can be demonstrated: **alpha (α) hemolysis** where the red blood cells are incompletely lysed and give off a green color around the bacteria colony whereas **beta (β) hemolysis** is the complete lysis, or destruction, of the red blood cell and produce a zone of clearing around the bacterial colony (Figure 2). No hemolytic reaction is often termed Gamma (γ) hemolysis.

A wide range of bacteria can be isolated with BA but the most common include the alpha-hemolytic *Steptococcus viridans*, *S. salivarius*, and *S. pneumonia while* beta-hemolytic bacteria include *Streptococcus pyogenes* and some strains of *Staphylococcus aureus*.

Table 1. Common bacteria isolated in this experiment from different sources

Source	Bacterium	Morphology	Catalase	Oxidase	Gram	Hemolysis
Skin	*Staphylococcus epidermis*	cocci	+	-	+	α
	Staphylococcus aureus	cocci	+	-	+	**β**
	Corynebacterium	rods	+	-	+	α
	Propionibacterium acne	rods	+	-	+	-
	Micrococcus luteus	cocci	+	+	+	γ
Mouth/ Throat	*Streptococcus pyogenes*	streptococci	-	-	+	**β**
	Moraxella c.	diplococcic	+	+	-	γ
	Streptococcus viridans	streptococci	-	-	+	α
	Neisseria sp.	cocci	+	+	-	γ
	Lactobacillus sp.	rods	-	-	+	α
Air	*Aerococcus*	cocci	-	-	+	
	Staphylococcus	diplococci	+	-	+	
	Bacillus	diplo, strepto	+	+/-	+	
	Micrococcus	cocci	+	+	+	

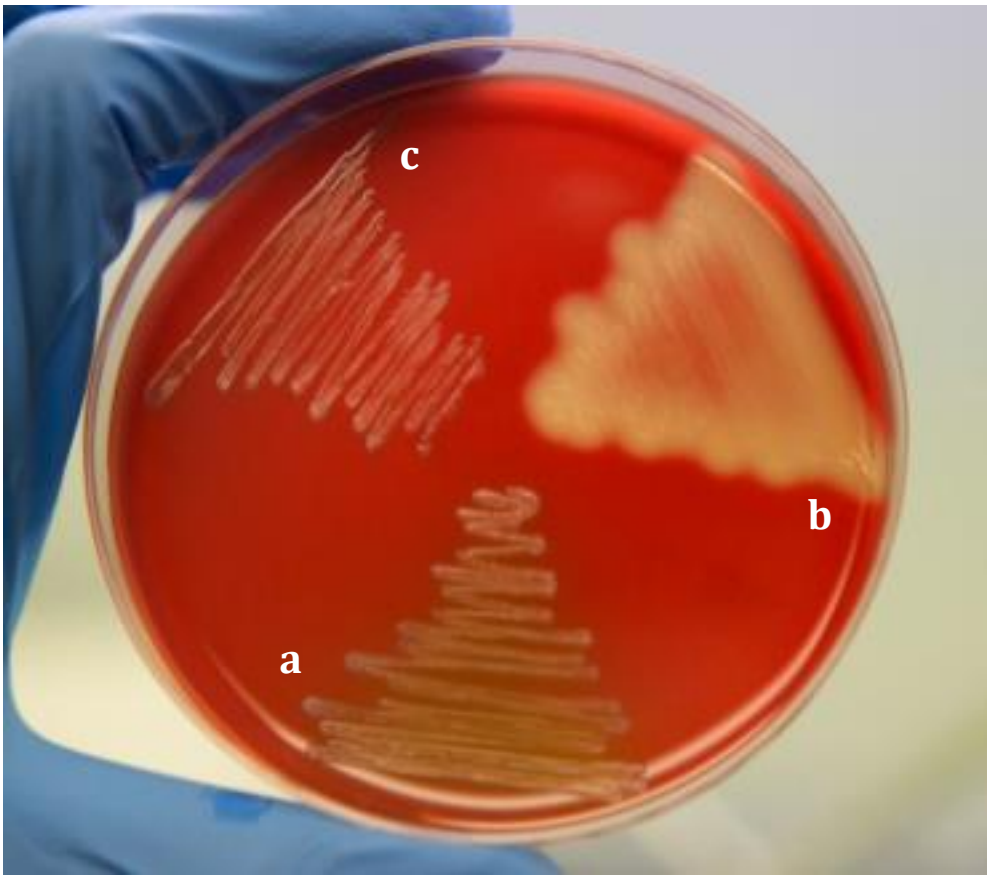

Figure 2. Blood agar showing three different bacteria inoculated into separate quadrants to show Alpha (a) Beta (b) and Gamma (c) hemolytic reactions

Materials Week 1:

1. **3 Nutrient Agar plates**
2. **1 Mannitol Salt Agar plates**
3. **1 Blood Agar plate**
4. **Sterile individually wrapped cotton swabs**
5. **1 tube of 0.85% NaCl (2ml)**

Materials Week 2:

1. **Catalase reagent**
2. **Oxidase reagent**
3. **Gram staining kit**
4. **Microscope slides**
5. **Loop**
6. **Stock cultures of 2 bacteria (*Staphylococcus aureus, Streptococcus pyogenes)***

Procedures for WEEK 1:

Part I. Air, skin and Nose Sampling

1. Obtain all your plates and make sure to label each with the proper media.

2. Obtain a Nutrient agar plate and label this plate “Air”. Expose it to the laboratory room by removing the lid until the end of the lab period. Incubate this plate at 30°C.

3. Using the sterile cotton swab, dip the swab into the tube containing the NaCl solution. Swab your hands, palms, and between your fingers making sure to rotate swab as you rub.

4. Streak the swab as a primary streak over one Nutrient Agar plate and one Mannitol salt agar labeled with “Skin”. Discard swab in biohazard.

5. Incubate both skin Nutrient and Mannitol salt agar plates at 37°C for 48 hours.

6. Carefully insert sterile swab into one nostril and gently swirl to collect wet sample. Be careful not to push too deep. Streak the swab as a primary streak onto a Nutrient Agar plate and Label this plate “Nose”. Incubate at 30°C for 48 hours.

Part II. Throat Swab

1. Obtain a tongue depressor and use to this to depress the tongue in order to swab the back of the throat. Use this swab to make a primary streak on a BA plate labeled “Throat”.

2. Use an inoculating loop to streak for isolation.

3. Incubate this plate at 37°C for 48 hours.

Procedures for WEEK 2:

1. Obtain all your plates and make sure for the following directions to OBSERVE each bacterial colony on the agar plates and record morphology in your worksheet.

2. Obtain your Nutrient Agar plate exposed to the air. On the back of this petri dish, mark and label TWO different bacteria colonies (Colony 1, Colony 2). Choose large colonies to have enough material to perform all tests.

3. Gram stain each colony, and perform the catalase and oxidase tests.

4. Obtain your four skin plates of Nutrient Agar and MSA plates. Count the number of bacterial colonies and colony types on each plate and record in your worksheet.

5. From the MSA plates, choose ONE bacterial colony that produced a yellow reaction for Gram staining and catalase and oxidase.

6. Observe you nose Nutrient agar plate. Pick one bacterial colony and gram stain, oxidase and catalase. Record in your worksheet.

7. Observe your BA plate for hemolytic reactions. Mark and label one alpha and one beta hemolytic reactive colony. Perform a Gram stain and catalase and oxidase test.

8. Record all your data into your worksheets.

Part I. Microscopic Observations

Directions: Record all your gram stain, oxidase, and catalase results with the proper bacterium.

Source	Colony Morphology	Sketch	Gram Stain	Cellular Morphology	Catalase /Oxidase
Air 1					
Air 2					
Nose					
Skin					
Throat α					
Throat β					

Part II. Skin Bacterial Count
Directions: Record the total number of separate bacterial colonies and the types (color, shape, texture) on all plate types exposed to bacteria from hands and nose.

	Nutrient Agar (Hands)	Mannitol Salt Agar	Nutrient Agar (Nose)
Total Number			
Total Types			

Part III. Questions

1. Explain and describe the main differences in morphology, gram stain, and catalase/oxidase reactions among the bacteria isolated from the air compared to bacteria isolated from the mouth and skin (hands/nose).

2. Taking the catalase/oxidase reaction and gram results into consideration and what you have learned from previous experiments, what types of bacteria are abundant in the human body?

3. How do the bacteria isolated from your mouth differ from your hands and nose bacteria? Use all of your results in order to draw your conclusions.

4. Using your results from Part II Skin bacterial counts; explain the difference you observe between the plates exposed to your hands including the Nutrient Agar (hands and nose) and the Mannitol Salt Agar. Why is there such a difference?

5. Using Table 1, Tentatively identify the bacteria you isolated from the various sources using your morphology, gram stain, and catalase and Oxidase reaction results.