EXPERIMENT 7

HUMAN SYMBIOTIC BACTERIA

Bacteria are common components of the human skin, throat, saliva as well as other parts of the body. In this experiment, we will isolate bacteria from your skin, throat and saliva and compare them with bacteria from air. These bacteria are considered the "normal flora" of the regions from which they will be isolated. These bacteria include pathogens.

This experiment will also utilize both **selective** and **differential media**. Selective media have ingredients (or conditions) that only allow for the growth of particular physiological types of microbes. Differential media contain ingredients that allow one to differentiate between different bacterial types based upon the colony morphology or reactions produced by the bacteria in the medium. **Mannitol Salt Agar** (Figure 1.) is both selective (contains 7.5% NaCl) and differential (contains the sugar mannitol and the dye phenol red). Thus only halophiles or halotolerant bacteria can grow on this medium and it allows the differentiation of colonies that can produce acids from mannitol because these will cause the medium surrounding the colony to become yellow....phenol red is red at neutral pH and yellow in acidic pH.

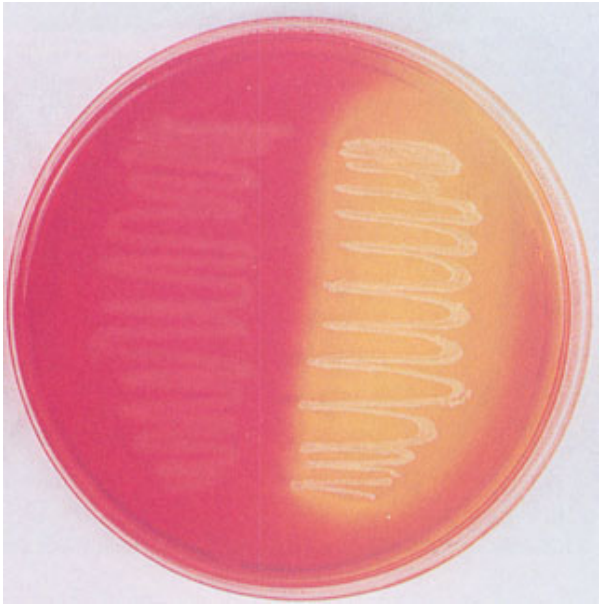


Figure 1. Different reactions produced by the bacterium in the medium (Red-neutral pH; Yellow-acidic pH)

Many severe human and animal infections can be caused by the heterotrophic bacteria carried on a normal individual. For example, hemolytic reactions can readily be demonstrated by growing these organisms on blood-agar plates. Two types of hemolysis can be distinguished: **alpha hemolysis** appears as a greening of the blood surrounding the colony and **beta hemolysis** is the complete destruction of the red blood cells forming a zone of clearing around the colony (Figure 2.). Alpha hemolytic bacteria include *Streptococcus viridans, S. salivarius and S.*

penumoniae. Beta hemolytic bacteria include *S. pyogenes*, and some strains of *Staphylococcus aureus*.

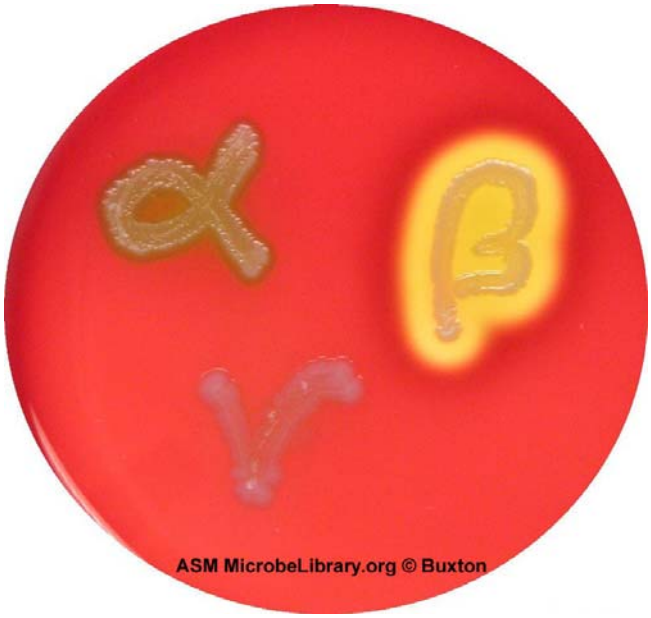


Figure 2. Differences between alpha and beta hemolysis

Although a wide range of bacteria can be isolated on the media used in this experiment, two major groups will most often be encountered: the aerobic cocci (*Staphylococcus* and *Micrococcus*) and the aerotolerant obligate fermentative Gram positive *Streptococcus*. Other frequently encountered bacteria may be *Neisseria* (Gram negative cocci), *Corynebacterium* (Gram positive, irregular rods) and *Bacillus* (Gram positive spore forming rods).

MATERIALS

First Lab Period

1. 2 Nutrient Agar plates.
2. 2 Mannitol Salt Agar plates.
3. 1 Blood Agar plate
4. 1 5% Sucrose-Nutrient Agar plate.
5. Sterile cotton swabs, individually wrapped.
6. 1 tube, 2 ml sterile 0.85% NaCl.
7. Stock cultures of *Staphyloccus aureus, Streptococcus pyogenes, S. viridans, S. lactis.*

Second Lab Period

1. Catalase Reagent.
2. Oxidase Reagent.
3. 1 Mannitol Salt Agar plate
4. 1 Blood Agar plate.
5. 1 5% Sucrose-Nutrient Agar plate
6. 1 2.5% Glucose+2.5% Fructose-Nutrient Agar plate
7. BHI slant cultures of *Pseudomonas aeruginosa* and *Escherichia coli* to demonstrate positive

and negative oxidase reactions.

Third Lab Period

1. 1 BHI slant

PROCEDURE

First Lab Period

1. **Air Plate**. Expose a Nutrient Agar plate to lab air for 30 minutes (with the top off). Cover and incubate at room temperature in an inverted position until the next period.

2. **Skin Swabs**. Dip a sterile cotton swab into a tube of sterile saline and above the saline, express the excess liquid out of the swab against the side of the tube. Swab a portion of your face, preferably in the area around the nose or forehead. Note that when swabbing skin, rotate the swab while wiping skin. This will capture the bacteria from skin as evenly as possible on the swab.

3. Streak the swab as a primary streak over each of a Nutrient Agar and a Mannitol Salt Agar plate. Discard the swab to a biohazard container.

4. Complete streaks for isolation with a sterile inoculating loop on each plate from the primary streak.

5. Go to a sink and wash the part of your face that was swabbed. Wash your face as you normally do, rinse and dry. Then with another sterile, moist cotton swab, swab the area of your face that was just washed. Use this to inoculate another Nutrient Agar plate and Mannitol Salt Agar plate. Complete the streaks for isolation using a sterile inoculating loop.

6. The instructor will prepare know Mannitol Salt Agar plates streaked with *Staphylococcus aureus* and *Micrococcus* sp.

7. Incubate the skin swabbed plates and the known cultures at 37^{o}C for 48 hours.

8. **Throat Swab**. Using a **dry** swab and a tongue depressor, swab your partner's throat. In doing this be careful not to contaminate the swab with saliva. Gently touch the rear of the throat and give the swab a short twist. Use this to make a primary streak on Blood Agar. Using a sterile inoculating loop, streak for isolation.

9. The instructor will inoculate Blood Agar plates with known cultures of *Streptococcus pyogenes, S. viridans,* and *S. lactis.*

10. Incubate the Blood Agar plates at 37^{o}C for 48 hours.

11. **Saliva Streak**. With a sterile and **cooled** inoculating loop, streak a portion of your saliva onto a plate of 5% Sucrose-Nutrient Agar. Sterilize your loop and then streak for isolation. Incubate the plate at 37^{o}C for 48 hours.

Second Lab Period

1. **Air Plate**. Mark and describe three different colonies on the plate exposed to air. Gram stain each colony. What are the predominate types of bacteria in air? Are there organisms other than bacteria in air?

2. **Skin Swabs**. Although we did not do a quantitative dilution and plating, compare the relative numbers of bacteria on each of the skin swabbed plates. Compare the numbers on Nutrient Agar with Mannitol Salt Agar and compare the plates before washing your face with after washing your face. Mark and describe the different colonies on the Mannitol Salt Agar plate and Gram stain each. Choose one (preferably a colony producing a yellow reaction in the medium) and restreak a Mannitol Salt Agar for a pure culture. Compare your colonies with the known colonies.

3. **Throat Swab**. Observe the Blood Agar plate for hemolytic reactions. Mark and describe hemolytic colonies and Gram stain representative alpha and beta hemolytic colonies. Compare your colonies with the known cultures.

4. Choose a hemolytic colony that appears to consist of only one cell type after examining your Gram stains. Using a sterile loop, restreak a Blood Agar plate with a hemolytic colony from your throat. Incubate at 37^{o}C for 48 hours.

5. **Saliva Streak**. Look for typical "dome shaped", clear colonies. Describe these colonies and Gram stain them. These colonies can often be difficult to work with because the capsule material makes the cells adhere tightly to each other; nevertheless remove part of the colony and smear vigorously in a small area on the slide.

6. Streak the remaining portion of the "dome shaped" colony onto each of 5% sucrose-Nutrient Agar and the Glucose-Fructose-Nutrient Agar. Be sure to streak each for isolation using aseptic technique.

7. **Oxidase Test**. After completing the observations above, perform the oxidase test on each organism described from air, your skin, your throat and saliva. To do the oxidase test, place a drop of oxidase test reagent on a slide, with a sterile loop remove part of the colony and place it into the reagent. If the cells turn blue within a minute it is a positive test.

8. **Catalase Test**. To the remaining of each colony described, add a drop of catalase test reagent (3% H_2O_2) and observe for bubbles. If positive, bubbles should appear within a couple minutes.

Third Lab Period

1. Examine the Mannitol Salt Agar and Blood Agar restreak plates. Are the colonies the same and the same as the originals? Gram stain each. After observing the Gram stains, choose one (either the skin or throat isolate) and streak a BHI slant. **Record** your results in the blank portions of the Results pages above. Incubate 24 to 48 hours and refrigerate. This isolate will be used later in the antibiotic assay (Experiment 13).

2. Observe the restreaks from the "dome shaped" colony from your saliva. Is there a difference

in colony morphology between the two different media? Write your observations in the blank space on the Results pages.

QUESTIONS

1. What advantages would a coccus shape have over a rod shape for survival in air?

2. How do bacteria cause the alpha and beta hemolytic reactions? How could this be related to pathogenicity? Could your answer to these questions be tested experimentally?

3. What is the use of a capsule for bacteria adapted to live in the human mouth?

RESULTS

Air Bacteria

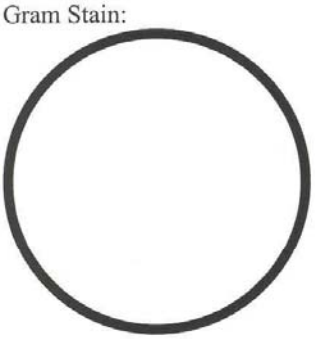
Colony 1

Colony Morphology:_____

_____

Catalase Test:_____ Oxidase Test:____

Gram Stain:



___X

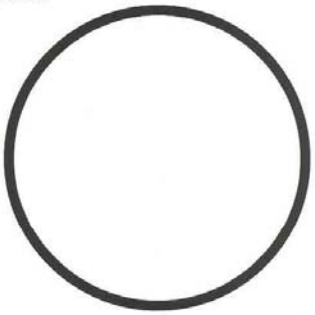
Colony 2

Colony Morphology:_____

_____

Catalase Test:___ Oxidase Test:___

Gram Stain:



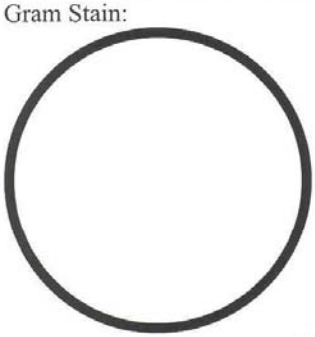
___X

Colony 3

Colony Morphology:_____

_____

Gram Stain:



___X

Catalase Test:____

Oxidase Test:____

Skin Swab:

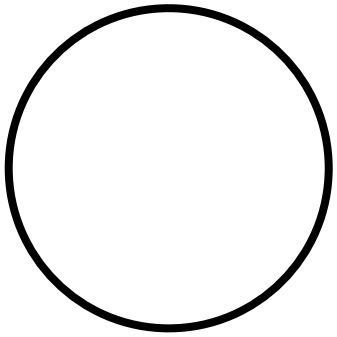
Relative Number of Bacterial Colonies on the Plates:

	Mannitol Salt Agar	**Nutrient Agar**
Before Washing:	_____	_____
After Washing:	_____	_____

Skin Bacteria:

Colony Description:_____
_____

Gram Stain:



_____X

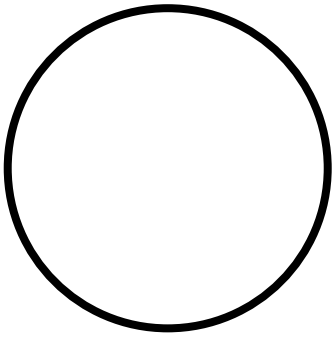
Catalase Test:_____

Oxidase Test:_____

Throat Swab:

Colony Description:_____
_____

Gram Stain:



_____X

Catalase Test:_____

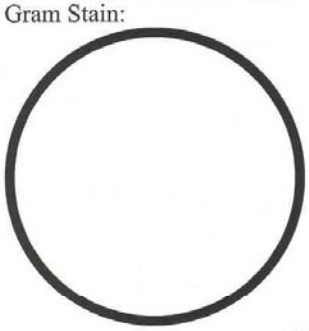
Oxidase Test:_____

Saliva Bacteria:

Colony Morphology:_____

_____

Gram Stain:



___X

Catalase Test:_____

Oxidase Test:_____

RESTREAKS

	Sucrose Nutrient Agar	**Glucose+Fructose Nutrient Agar**
Colony Description:	_____	_____

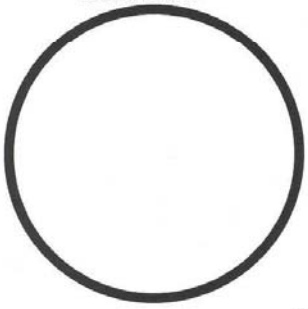
Restreaks to Obtain a Pure Culture:

Medium/Colony Resreaked:_____ (MSA or Blood Agar/Colony)

Restreak Plate: Colony Morphology:_____

_____

Gram Stain:



___X