THE IDENTIFICATION OF TWO UNKNOWN BACTERIA

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TEST TUBE 3
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Introduction: The identification of bacteria is important in order for us to differentiate one microorganism from another and classify them based on their morphology, ecology, genetics, and most importantly by species. Knowing bacterial species is especially useful because we can determine how different bacteria affect our existence, as some bacteria may be disease causing while others may be beneficial to the wellbeing of humans and our ecosystems. As a result, the identification of bacteria is very significant in the medical and pharmaceutical industries.

The purpose of this experiment was to use techniques learnt throughout the semester to successfully identify two unknown bacteria, one gram-positive and one gram-negative, mixed in a broth culture in test tube 3.

Materials and Methods: The methods below were followed from those listed in the course laboratory manual.

An unknown bacterial culture in a test tube labeled 3 was chosen. Using aseptic techniques, streak plates were made and incubated for two days each on the following plates:

- Blood Agar (BA) to determine the hemolytic properties of both unknown bacteria
- MacConkey Agar (MAC) that will select for the gram-negative bacterium and also determine whether or not it is a lactose fermenter. Colonies produced on this plate will also be used for biochemical testing to determine its identification.
- Mannitol Salt Agar (MS) that will determine if the unknown gram-positive bacterium is of the *Staphylococcus* species and if so, whether or not it ferments mannitol.
- Phenylethyl Alcohol Agar (PEA) that will select for the gram-positive bacterium.

- Nutrient Agar to observe how the bacteria grow and the color of their colonies.

Using gram-staining and aseptic techniques, a gram stain was prepared with a sample from the broth culture and observed under the microscope.

Following the incubation period, the BA, MAC, MS, PEA, and NA plates were observed to determine which biochemical tests will be performed. The biochemical tests subsequently performed, using colonies from the MAC plate, were as follows:

- Methyl-Red Voges-Proskauer (MR-VP)
- Citrate
- Triple Sugar Iron Agar (TSIA)
- Sulfur Indole Motility (SIM)

A second gram stain was then performed using a colony from the NA plate and observed under the microscope.

Results: Figures 1 through 5 below are pictures of the results obtained from the inoculated streak plates. Figure 6 is a picture of the second gram stain obtained from nutrient agar.

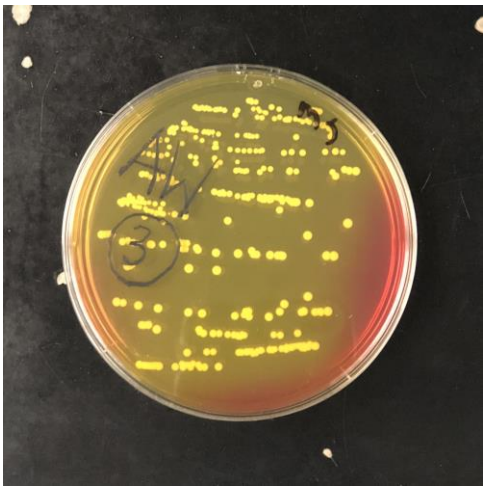

Figure 1: Mannitol Salt Agar

Figure 2: Blood Agar

Figure 3: MacConkey Agar

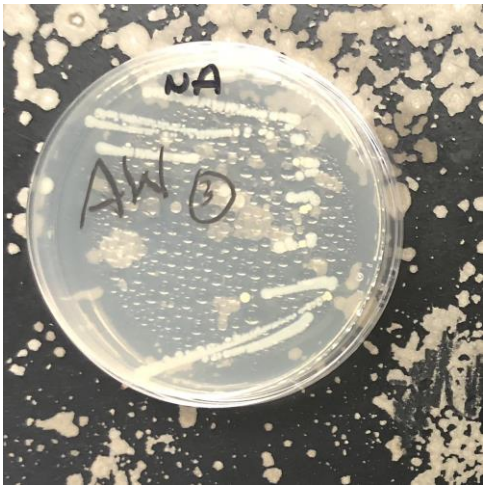

Figure 4: Nutrient Agar

Figure 5: Phenylethyl Alcohol Agar

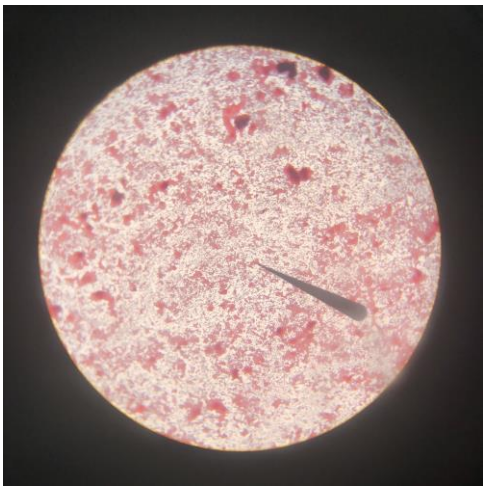

Figure 6: Gram Stain from NA plate

Figures 7 through 10 are the results from biochemical testing.

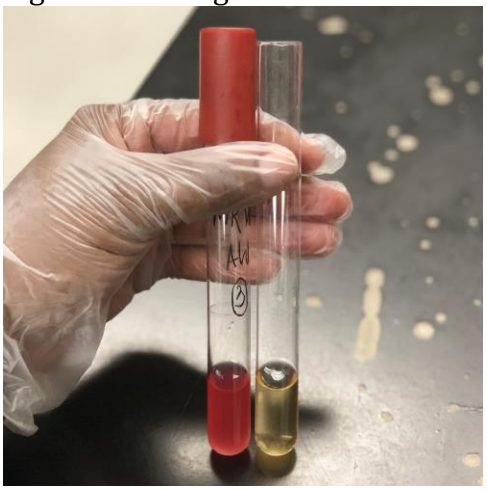

Figure 7: MR-VP

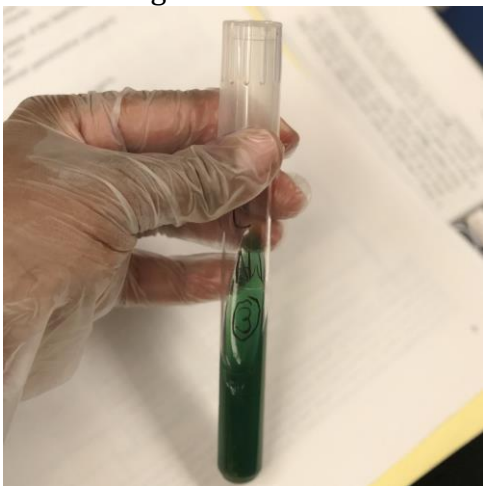

Figure 8: Citrate

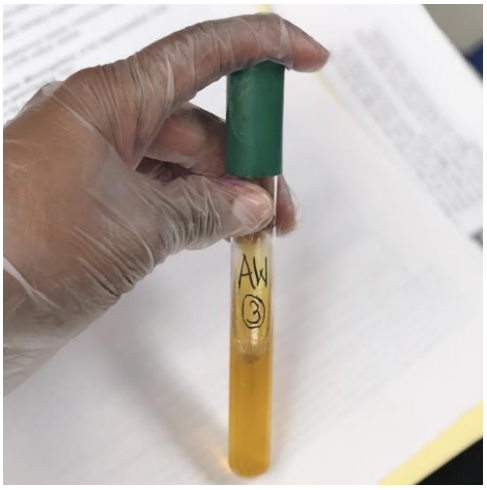

Figure 9: TSIA

Figure 10: SIMS

The charts below were prepared and used as references throughout the experiments:

Chart 1: Gram Negative Bacteria and Their Characteristics

Characteristics	Enterobacter aerogenes	Escherichia coli	Pseudomonas aeruginosa	Proteus vulgaris	Serratia Marcescens
Gram Staining	Gram Negative	Gram Negative	Gram Negative	Gram Negative	Negative
Shape/Color	Rod / Whitish	Rod / Whitish	Rod / Grey/Greenish	Rod / Greyish	Rod / White/Pink/or Red
Motility	Positive	Positive	Positive	Positive	Positive
Capsule	Capsulated	Capsulated	Capsulated	Non-Capsulated	Negative
Spore	Non-Sporing	Non-sporing	Non-Sporing	Non-Sporing	Non-Sporing
Acid-Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast
BA	Gamma	Beta	Beta	Beta (Swarms)	Gamma
MS	No Growth	No Growth	No Growth	No Growth	No Growth
MAC (- only)	Growth/Fermentor (pink colonies)	Growth/Fermentor (pink colonies)	Growth/Non-Fermentor	Growth/Non-Fermentor	Growth (red colonies)/Non-Fermentor
PEA (+ only)	No Growth	No Growth	No Growth	No Growth	No Growth
DNase	Negative	Negative	Negative	Negative	Positive (Halo)
Starch Hydrolysis	Negative	Negative	Negative	Negative	Negative
Gelatin Hydrolysis	Negative	Negative	Positive	Positive	Positive
Glucose	Positive (Yellow)/(AG)	Positive (Yellow)/(AG)	Negative (Orange)/Alkaline	Positive(Yellow)/(AG or A)	Positive (Yellow)/(AG or A)
Lactose	Positive (Yellow)/(AG)	Positive (Yellow)/(AG)	Negative (Orange)/Alkaline	Positive (A/AG) or Negative	Positive(Yellow) or Negative(Orange)
TSIA (Slant/Butt)	ACID(Yellow)/ACID(Yellow)/Gas	ACID(Yellow)/ACID(Yellow)/Gas	ALK (Red)/ ALK	ALK(Red) /ACID (Yellow)/H2S	ALK(Red) / ACID(Yellow)
Catalase	Positive (Bubbles)	Positive (Bubbles)	Positive (Bubbles)	Positive (Bubbles)	Positive (Bubbles)
Oxidase	Negative (Colorless)	Negative (Colorless)	Positive (Purple)	Negative (Colorless)	Negative (Colorless)
MR	Negative (Yellow)	Positive (Red)	Negative	Positive (Red)	Negative (Yellow)
VP	Positive (Pink/Orange)	Negative (no color change)	Negative (no color change)	Negative (no color change)	Positive (Pink/Orange)
Indole	Negative (Yellow)	Positive (Red)	Negative (Yellow)	Positive (Red)	Negative (Yellow)
Citrate	Positive (Blue)	Negative (Green/no color change)	Positive (Blue)	Negative (Green/no color change)	Positive (Blue)
Litmus Milk	ACID (Pink)	ACID (Pink)	DIGESTION (Becomes Clear)	ALKALINE (Blue)	REDUCTION (White)
Urease	Negative (Yellow)	Negative (Yellow)	Negative (Yellow)	Positive (Pink)	Negative (Yellow)
Nitrate Reduction	Positive	Negative (turned red)	Positive (Gas)	Positive	Positive
H2S	Negative	Negative	Negative	Positive (Black)	Negative
Gas	Positive (Split Agar)	Positive (Split Agar)	Positive (From Nitrate Reduction)	Negative	Sometimes

Chart 2: Gram Positive Bacteria and Their Characteristics

Charateristics	Bacillus subtilis	Bacillus stearo - thermophilus	Clostridium sporogenes	Staphylococcus epidermidis	Staphylococcus aureus	Enterococcus faecalis	Micrococcus luteus	Mycobacterium smegmatis
Gram Staining	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Shape/Color	Rod / White	Rod	Rod	Cocci(Clusters) / White	Cocci(Clusters) / Yellowish	Cocci(Chains) / Greyish	Cocci(Tetrads) / Yellow	Rod
Motility	Motile	Motile	Motile (Flagella)	Non-Motile	Non-Motile	Non-Motile	Non-Motile	Non-Motile
Capsule	Non-capsulated	Capsulated	Non-capsulated	Mostly capsulated	Non-capsulated	Capsulated	Non-capsulated	Capsulated
Spore	Sporing	Sporing	Positive	Non-sporing	Non-sporing	Non-Sporing	Non-sporing	Non-sporing
Acid-Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast	Non-Acid Fast	Non Acid-Fast	Non Acid-Fast	Acid-fast
BA	Beta	Beta	Beta	Gamma	Beta	Gamma	Beta / Vivid Yellow	Gamma
MS	Growth (Non-fermentor)	Growth (Non-fermentor)	Growth (Non-fermentor)	Growth (Non-fermentor)	Growth (yellow halo)	Growth	Growth (Non-fermentor)	Growth (Non-fermentor)
MAC (- only)	No Growth	No Growth	No Growth	No Growth	No Growth	No growth	No growth	No growth
PEA (+ only)	Growth	Growth	Growth	Growth	Growth	Growth	Growth	Growth
DNase	Positive	Positive	Positive	Negative	Positive	Negative	Negative	Negative
Starch Hydrolysis	Positive		Negative	Negative	Negative	Negative	Negative	Negative
Gelatin Hydrolysis	Positive		Positive	Negative	Positive	Negative	POS OR NEG	Negative
Glucose	Positive	Positive	Positive	Positive A/G	Positive A/A	Positive A/AG	POS OR NEG	Negative
Lactose	POS OR NEG	Negative	Negative	POS OR NEG	Positive A/A	Positive A/AG	Negative / ALK	Negative
TSIA (Slant/Butt)	ALK (Red) / ACID (Yellow)			ALK/ACID	ACID/ACID	ACID/ACID	ALK/ALK	ALKALINE
Catalase	Positive (Bubbles)	Positive (Bubbles)	Negative	Positive	Positive	Negative	Postive	Positive
Oxidase	Positive (Purple)	Positive (Purple)	Negative	Negative	Negative	Negative	Positive	Negative
MR	Negative (Yellow)		Positive (Red)	Negative	Positive (Red)	Positive (Red)	Negative	Negative (Yellow)
VP	Positive		Negative	Negative	Positive	Negative	Negative	Negative
Indole	Negative		Negative	Negative	Negative	Negative	Negative	Negative
Citrate	Positive			Negative	Positive	Negative	Negative	Positive
Litmus Milk	ALKALINE		ACID/CLOT	Alkaline	ACID	ACID	Alkaline	Alkaline
Urease	Negative	Negative	POS OR NEG	Negative	Negative	Negative	Weak Positive	Negative
Nitrate Reduction	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Positive
H2S	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative
Gas	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative

Discussion:

The initial gram stain prepared from the tube 3 broth was unsuccessful and may have been due to experimental error during preparation. The second gram stain prepared using a colony from the nutrient agar plate showed that one of the bacteria was a gram-negative rod (Figure 6). This test was deemed useless for identification because all the gram-negative bacteria studied were rods.

The unknown gram-positive bacterium was identified as *Staphylococcus aureus*. This identification was deduced based on the results from the blood agar plate and confirmed by the Mannitol Salt Agar plate. From the blood agar plate (Figure 2), I was able to observe that both bacteria displayed beta hemolytic properties due to the clearing around the colonies. Mannitol Salt Agar however, is a medium that is selective for the *Staphylococcus* species because they are halophiles, that is, they can tolerate high salt concentrations. As a result I was able to identify that the gram-positive bacterium present in tube 3 was a

Staphylococcus because there were colonies present in the MS plate. MS is also a differential medium used to identify mannitol fermenters that will produce acid and turn the medium yellow. Based on this knowledge, I was able to determine that the gram-positive bacterium was *Staphylococcus aureus* because of the two *Staphylococcus* species studied, it is the mannitol fermenter and the MS plate was almost completely yellow after the incubation period as seen in Figure 1 of the results. No further testing was necessary for identification.

The unknown gram-negative bacterium was identified as *Escherichia coli*. MacConkey Agar was first used to isolate the gram-negative bacterium because it inhibits the growth of gram-positive bacteria. MAC is also differential in identifying gram-negative lactose fermenters that produce a pink color. Following the incubation period the results as seen in Figure 3 determined the bacterium was a lactose fermenter. Through this observation and based on the gram-negative bacteria studied, I was able to narrow down my gram-negative bacterium to *Enterobacter aerogenes*, *Escherichia coli*, or *Serratia marcescens*. After observing my nutrient agar plate (Figure 4), I did not think the bacterium was *Serratia marcescens* because it would have grown as reddish-brown colonies. The blood agar plate (Figure) also led me to believe that the bacterium was *E. coli* because of the beta hemolytic action observed. However, the MR-VP, TSIA, SIMS, and citrate biochemical tests were used for further identification because I was still uncertain. The ACID/ACID results from the TSIA (Figure 9) allowed me to safely eliminate *S. marcescens* because it is ALKALINE/ACID. The remaining test results were all characteristic of *E. coli* with the exception of the indole test which was negative when performed in the SIMS tube. This may have been due to cross contamination upon inoculation. The negative citrate test (Figure 8), and positive MR/ negative VP tests (Figure 7) results were however used to successfully identify the gram-negative bacterium as *Escherichia coli*.

Staphylococcus aureus, according to the Centers for Disease Control (CDC), is a bacterium that resides in the noses of approximately 30% of the human population. It also can be found in the skin and gastrointestinal tract. Majority of the time *S. aureus* is not harmful but in healthcare settings however it may become infectious and even lead to death. It can travel into the bloodstream of individuals causing sepsis or bone infection. *S. aureus* is also known to be resistant to certain antibiotics which include Methicillin and Vancomycin. Individuals that are often at risk for staph are those with conditions like diabetes, eczema, and cancer.

Escherichia coli are a diverse group of bacteria that are most often harmless. It is normally found living in the intestines of healthy individuals. Some strains of *E. coli* however can be infectious as a result of consuming contaminated food or water and can cause severe abdominal pain, excessive vomiting, and bloody diarrhea according to the CDC. According to Foodsafety.gov, the most toxic strain of *E. coli* is O157:H7, which produces the Shiga toxin and can cause kidney failure and death. *E. coli* was also the first bacterium used to study the stringent response in bacteria.

References

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