Identification of an Unknown

Unknown number 5

Asheleee Barreau

Professor Haque Nasreen

Bio 3302-E178

Introduction: The importance of bacteriology, that is, the study of bacteria is absolutely undeniable. This branch of biology has made it possible to identify and classify different bacteria. Through the study of bacteria, we are able to analyze the "personality" of an bacteria, which the characteristics such as morphology and biochemistry of a particular bacteria. This branch of biology has also made it possible to distinguish one organism from another. In this way, we are able to distinguish between non-disease causing and disease causing, that is, pathogenic bacteria. The tests and techniques used to study bacteria are essential to the identification process. The purpose of this experiment was to perform various tests and techniques, learned throughout the course of this semester, in order to both distinguish between the gram negative and positive bacteria in test tube #5 and to correctly identify both bacteria in this test tube.

Materials and Methods:

In order to identify the unknown bacteria in test tube #5, a gram stain was done followed by the streaking of plates using aseptic techniques in order to grow pure isolated colonies, followed by biochemical testing.

- Gram Staining was used to distinguish the gram positive bacterium from the gram negative bacterium.
- Nutrient Agar is used to grow pure cultures in order to clearly observe the characteristics of a bacteria: morphology, color, odor, and etc.
- Blood Agar (BA) is used to determine the hemolytic properties which could potentially help distinguish between both unknown bacteria.

- MacConkey Agar (MAC) allows gram negative bacteria to grow by inhibiting the growth of gram positive. This medium also aides in the differentiation between non-lactose fermenters and lactose fermenters.
- Mannitol Salt Agar (MS) allows the gram positive, halophilic organisms which are of the *Staphylococcus* species to grow, while inhibiting the growth of both gram-negative and non-salt tolerant bacteria.
- Phenylethyl Alcohol Agar (PEA) is selective, that is, allows for gram-negative bacteria to grow.
- Citrate tests an organism's ability to use carbon as its only carbon source.
- Triple-Sugar Iron Agar (TSIA) is used to identify gram negative enteric bacteria, it tests an organisms ability to ferment selected carbohydrates and produce hydrogen sulfide.
- Catalase determines which organisms have the enzyme that breaks down hydrogen peroxide (H202). Catalase is useful in distinguishing between gram negative and positive cocci.

Results:

Figure 1: Nutrient Agar

Figure 2: Blood Agar

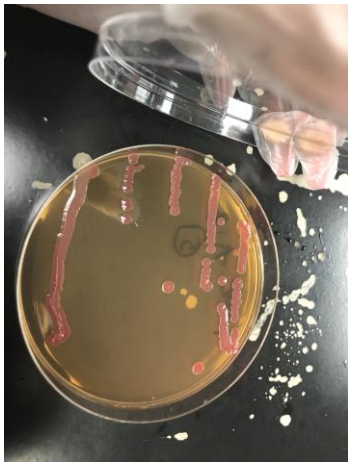

Figure 3: MAC

Figure 4: Mannitol Salt

Figure 5: PEA

Figure 6: Citrate Tube 1

Figure 7: Citrate Tube 2

Figure 7: TSIA Tube 1

Figure 8: TSIA Tube 2

Figure 8: Catalase

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

Figure 9: Gram Negative Chart

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

Figure 10: Gram Positive Chart

Discussion/Conclusion: I concluded that in tube #5 was gram-positive bacteria Bacillus subtilis and gram-negative bacteria *Serratia marcescens*. My gram-stain, which I attempted to do several times, was unsuccessful each time maybe due to experimental error during the preparation process and so was unshelpful in my identification process. To test my hypothesis, I ran several biochemical tests. In figure 1, some of the colonies that grew on the nutrient agar were red. Red colonies also grew throughout the MacConkey agar, which led me to conclude that my gram negative bacterium was *Serratia marcescens*. To further support this conclusion, I did the citrate test by inoculating a gram negative colony from the MAC agar then stabbing into the citrate test tube. I expected to have a positive result which would cause the control which was green to turn blue. As I expected, for *S. marcescens*, a positive reaction occurred as shown in figure 6. I also inoculated a colony from the MAC agar into a Triple-Sugar Iron Agar test tube, by stabbing the

medium. As I expected, the slant turned red which means alkaline (proteins) were catabolized, while the butt remained yellow from the control which means acid was produced from glucose fermentation (figure 8). In figure 8, a little bit of black precipitate which indicates hydrogen sulfide production was also observed, however this was not expected and could have happened due to extended incubation. To support my conclusion that my gram-positive bacteria was *Bacillus subtilis*, I used the mannitol salt agar. I used this agar because I knew that only organisms of the *Staphlycoccus* species would grow on this medium. As shown in figure 4 and as expected, there is no growth of bacteria throughout the entire medium; the little bit of growth present is possible indication of contamination that most likely occurred while I was inoculating a colony from the nutrient agar. For this bacteria, I also did the citrate test as shown in figure 6, which as I expected resulted in a positive reaction (tube turned blue). However, some green is still seen in the bottom of the tube because I did not stab the butt all the way. I also performed the TSIA test for this bacteria also, as shown in figure 7. For *B. subtilis*, as I expected, the slant turned red while the butt remained yellow. Similarly, to *S. Marcescens*, after metabolizing glucose and producing acid, *B.subtilis* begin to break down protein. In addition to these two test, I did a catalase test, as shown in figure 8 and as I expected *Bacillus subtilis* was positive for catalase which resulted in bubbling. Through these biochemical tests, I was able to make an accurate analysis of both bacteria.

Each of these bacteria has its own clinical significance. *Bacillus subtilis* which is mainly found in the soil, is non-pathogenic and is clinically important to identify because they are endospore forming. According to Microbe Wiki, during sporulation, *Bacillus subtilis* can form antibiotics. On the other hand, *Serratia marcescens* is clinically significant because it is an opportunistic nosocomial (hospital acquired) pathogen. According to Antimicrobe, it is not

usually the primary cause of infection but it acts as a true opportunist once the immune system is already compromised making it suitable for this bacteria to gain access. This bacterium affects patients that are dealing with debilitating and immunocompromised disorders. *S. marcescens* is implicated in a wide range of infections such as infections dealing with the respiratory tract, urinary tract, bloodstream infections, and wound infections. It is important to know why these bacteria are clinically significant, in order to understand what one is working to treat in the health care field.

Citation Page

Obenauf, S. (2017). *Microbiology Fundamentals: A Clinical Approach* (2nd ed.). United States of America: Mcgraw-Hill Education.