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
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 unhelpful 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.