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Kimsey Cooper
Microbiology 210 Lab
Final Unknown Report
Section#006/ Radha

Report on Identification of Mixed Culture Unknown
Lab Exercise 33

Introduction

The purpose of this experiment is to apply the knowledge gained from the entire semester in the Microbiology Lab and apply it to be able to identify bacteria. Each student was given a tube that had a mixture of two different types of bacteria inside. The tube used in this experiment was tube number fourteen. Inside the tube was one gram negative and one gram positive organism. The bacteria of which we learned about and of which were possibilities to be inside the test tubes include: *Corynebacterium xerosis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Clostridium perfringens*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Neisseria flavescens*, *Proteus vulgaris*, and *Moraxella catarrhalis*. The organisms in the test tubes must be streaked for isolation for oxygen requirements and followed with additional tests based on results.

Materials and Methods

The first steps taken in lab were to conduct a gram stain from the organisms in the mixed culture using the techniques and steps in exercise seven. Using aseptic techniques from exercise 5 in the lab manual, I then inoculated the mixed culture onto two Tryptic Soy Agar, TSA, plates and incubated them for 24 hours in 37C in both aerobic and anaerobic conditions. The next week, I aseptically streaked the mixed culture from the tube again with an inoculating loop onto two different types of agar. One agar used was Columbia CNA agar with 5% sheep's blood for gram positive organisms and the other agar was MacConkey agar, MAC. I inoculated both plates for 24 hours in 37C. After that, I aseptically conducted another gram stain test using lab exercise seven to be sure of the morphology of my organisms. On the CNA agar I checked for hemolysis and on the MAC agar I checked for a difference in colony color. The next route I took was for gram positive organisms.

For the gram positive organisms I first conducted a Carbohydrate Fermentation and Hydrogen Sulfide production test. I used Triple Sugar Iron, TSI, agar slants. I aseptically streaked the top of the agar slant and stab inoculated the agar 1/4 of the way down. I then incubated it for 24 hours at 37C. The next test I conducted was the Catalase test. For this test I needed two clean glass slides and a bottle of 3% H₂O₂. I aseptically mixed a bacterial colony onto a clean glass slide with a drop of water. Then I put one or two drops of 3% H₂O₂ directly on top of the organism and watched for bubbling. Another

Abstract

Dr. Maxwell T. R.

28 March 2010

In each test, we examined two different ways to control microbial growth in a lab setting. The two ways we used to control microbial growth were exposing the microorganisms to heat and UV light for a certain amount of time. Once we had completed exposing each group to the predetermined of time, we incubated for 48 hours. The two organisms we used for the experiments were *Escherichia coli* and *Bacillus subtilis*. Our results for the first part of the lab, were based on putting microorganisms in a hot bath for a certain time and temperature. After the incubation, we examined the growth for each test tube, which was compared a different temperature and time it was in the bath.

We can conclude from our data we thought they were both in the bath for two minutes, *E. coli* takes a higher temperature to be killed. This is because it is more difficult to heat from producing more endospores. Another way we tried to kill bacteria was using UV light. In the control by heat experiment, there was growth in one tube and no growth in the other, growth in both tubes, or no growth at all in either tube. The organisms that were used were *E. coli* and *B. subtilis*. In my individual test tubes, there was growth in the test tube that contained *B. subtilis* and no growth in the test tube that contained *E. coli*. My temperature was 80 degrees C, for 20 minutes. In greater part of the amount of the test tubes with different temperature and time, there was growth. The thermal death point is the lowest temperature needed to kill all microbes at a given time is 10 minutes (Davis, 2008). The thermal death time is the minimal time necessary to kill all bacteria present in the culture at a given temperature (Davis, 2008). For *E. coli*, the thermal death point is 50 degrees C, and the thermal death time is 10 minutes. *E. coli* has no growth for both 80 and 100 degrees C, at the different times. For *B. subtilis*, the thermal death point is 80 degrees C, and the thermal death time is 10 minutes. These organisms did not all grow at the same time and temperature. However, they did have growth at some time. In the results, both organisms had increased at 80 degrees C and 100 degrees C at each various temperature.

For the part of the experiment, we exposed UV light at different lengths of time to *E.*

UNKNOWN LAB REPORT

Unknown Number 115

Introduction

The process of identifying bacteria is like solving a mystery; all requiring is to identify the clues. Each clue will offer possibility to solve the puzzle. Bacteria were among the first life forms on Earth and are present all around from the bottom of the ocean to inside the human body. Bacteria come in all shapes and sizes. Although only a few micrometers in length, bacteria can still be examined through the use of a simple light microscope. The varying characteristics of bacteria play a crucial function in their identification (Hogan, 2010).

There are multiple laboratory techniques such as streak isolation, Gram staining, Catalase, or Simmon's Citrate that can be used to identify a bacteria. For

Identification of unknown bacteria lab report introduction.

Unknown Lab Report: Unknown "C" Work Cited"E. The body generally clears the bacterial infection on its own within 7-10 days. aeruginosa is what is called an opportunistic human pathogen, because it rarely affects a healthy individual. The second unknown bacteria was identified as a gram positive bacteria with a coccus shape. coli. This was done to ensure a pure and fresh sample of the unknown for the next week. This will be a vital task to take with me into my profession for many reasons. It is also important in knowing and understanding which microbes are beneficial to a person and important to the human body functions. Print. References: McDonald, Virginia, Mary Thoele, Bill Salsgiver, and Susie Gero. In order to do this, a nutrient agar plate was used. In order to do the streak method, an inoculating loop was sterilized with a Bunsen burner and put into the unknown specimen. At this point everything that had been learned in microbiology lab and that had been explained in our lab manual (1) was put into action. The gram negative bacteria in unknown #123 is a very aggressive bacteria that makes the growth of a gram positive bacteria difficult. Materials and Methods: The unknown number 123 handed out by the Professor on March 20, 2014 contained both a gram positive bacteria and a gram negative bacteria. coli infections; instead, sufferers are instructed to rest, stay hydrated, and to abstain from treating with an anti-diarrheal. These bacteria must be able to be identified in order to treat patients properly, efficiently and safely. After getting a good gram stain the identification tables were referred to in order to choose between appropriate biochemical tests. "Health Care Associated Infections." Centers for Disease Control and Prevention. This immediately ruled two of the gram positive bacteria Bacillus cereus and Bacillus subtilis. aeruginosa are those in hospital settings, especially ones on breathing machines or with catheters (3). "P. Some kinds of E. A sample was taken from the isolated nutrient agar and a gram stain was done as directed by the lab manual (1). Returning to the original unknown stock 123, a quadrant streak plate was done with a sterilized inoculating loop on a mannitol salt agar, which inhibits the growth of gram negative bacteria. The second test performed was a Urea Test which also gave a clear positive result confirming that unknown gram positive bacteria as Staphylococcus epidermidis because Staphylococcus aureus gives a negative result. A nitrate test was performed in order to detect if the bacteria was able to reduce nitrate into nitrite or some further reduced form. It builds resistance against a lot of antibiotics and even chemotherapeutic agents. It is most dangerous to cystic fibrosis patients and is involved and complicates 90% of cystic fibrosis deaths. To avoid the spread and contamination of P. Consultation with the Professor, confirmed the two unknown bacteria correctly as Pseudomonas aeruginosa and Staphylococcus epidermidis. The two bacteria that give a positive result for this test are Staphylococcus aureus and Staphylococcus epidermidis. 22 Apr. Table 2: Tests and Results for Gram Positive Bacteria Test Purpose Reagents or Media Observations Results Gram Stain To determine if the bacteria was gram negative or gram positive Crystal violet, Iodine, Alcohol, Safranin Purple coccus Gram Positive Cocci Nitrate To determine if the bacteria reduced nitrogen to some further reduced form such as nitrite Nitrate Reagents A & B, Zinc When reagents A & B were added the broth turned red Positive, the bacteria reduced the nitrate into nitrite or some further form of nitrogen Urea To determine if the bacteria produced the enzyme urease Urea Broth (yellow) Broth turned a hot pink color Positive, the bacteria produced the enzyme urease Discussion/Conclusion The unknown #123 contained two different specimen of bacteria, one being a gram positive bacteria and one being a gram negative bacteria. St. Louis: St. Louis Community College at Meramec, 2011. On 29 May 2017, the first procedure to be done was to streak the unknown out on a nutrient agar plate. aeruginosa nurses and health care professionals should be sure to use aseptic technique because the bacteria is mostly spread through contaminated equipment and professionals hands. Then a urea test was performed to check for the production of urease. Unknown C was determined to be a Gram- negative rod. All of the biochemical tests performed were explained, in the lab manual provided by Professor (1) and were practiced earlier in the semester. The streak method was used to spread the bacteria across the nutrient agar in hopes of isolating a pure culture of one of the bacteria. After removal with bacteria on the loop, the quadrant streak method was used. The first step to figuring out the unknowns, was to separate the two bacteria. His study picked up on the unique blue-green pigmentation of P. Pseudomonas aeruginosa is a gram negative rod shaped bacteria that was first discovered in 1882 by a pharmacists named Carle Gessard (2). cdc/ecoli/general/index.html By CPR Louisville at June 19, 2014 | 5:55 pm | Print Example of a Microbiology Unknown Lab Report by Taylor Autry Introduction In this paper I will discuss the processes of how I came to find my two unknown bacteria. Results The first test performed on the gram negative bacteria, was a Casein Test. Lab Manual for General Microbiology. All procedures were followed as stated in the course laboratory manual and also as further elaborated on by Professor Woolam. All other tests conducted went well except for the Endospore Stain which resulted in a false positive for the presence of endospores. 2013. This test gave a positive result turning a brown color, meaning the gram negative bacteria produced the enzyme casease in order to break down the milk protein casein. After inoculation of the nutrient agar plate, it was labeled, sealed, and placed aside for incubation and growth for 7 days. Tables 1 and 2 lists the tests, purposes, reagents used and the results of each test. P. From previous biochemical tests done in the semester. Pseudomonas aeruginosa was already suspected because of the green pigment of the original streak plate. The gram stain showed a result of red, gram negative rods. Centers for Disease Control and Prevention. 02 Apr. coli (Escherichia coli)." Center of Disease Control. The next test performed was a Casein Test which showed a clear positive result. The next test performed was a Nitrate Test which gave a positive result. Web. coli are harmless, others can make you sick. Unknown Lab Report: Unknown "C" IntroductionIt is important to know the identity of microorganisms for knowing how bacteria works and how it is structured means knowing how it can affect humans. It is not recommended to use antibiotics for the treatment of E. The streak plate that was originally done, showed a heavy green pigment, which is a main characteristic of Pseudomonas aeruginosa. Table 1: Tests and Results for Gram Negative Bacteria Test Purpose Reagents or Media Observations Results Gram Stain To determine whether the bacteria was gram negative or gram positive Crystal violet, Iodine, Alcohol, Safranin Red Rods Gram Negative bacteria Casein To determine if the enzyme casease was produced to break down the milk enzyme casein Milk Agar (white opaque) Milk Agar changed color where bacteria was smeared, turning a brown color Positive, the bacteria produced casease The first test performed on the gram positive bacteria was the Nitrate Test which turned red after adding reagents giving a positive result meaning the bacteria reduced nitrate into nitrite or something further. 23 Apr. This bacteria more so affects individuals with compromised immune systems the most common being those with cystic fibrosis, cancer, or AIDS. cpr louisville articles, cpr louisville, disease The purpose of this study was to identify the unknown bacteria by applying all the methods that have been learned so far in the microbiology laboratory class. 2016. A person can contract E. The gram stain procedure was performed as directed in the lab manual (1). To decipher between which biochemical tests to perform, the gram positive and negative tables handed out by the Professor, were referred to. Upon returning and observing the streak plate, there was an abundance of green across the plate. aeruginosa. There was only one colony that was apparent. Escherichia coli are bacteria naturally found in the intestines of humans and animals. According to the Center of Disease Control (CDC), "Although most strains of E. The gram stain showed clear purple gram positive cocci. All methods that have been learned so far for identifying bacteria have been applied to this unknown. Following the preparation of the inoculated nutrient agar plate, a heat fixed slide was prepared and a Gram stain was performed. This observation was brought to the attention by Professor Woolam and was quickly confirmed personally. This was determined by observing that all "positive" spores were located outside of the bacterial cells and none were located within them. Upon return and observation, the MSA did not yield a good isolated colony. This identification was reached by only one biochemical test and close observation. By knowing which agents are causing a disease, one can determine the correct treatment necessary with persons afflicted with the microbes in question. 2014. aeruginosa is quickly on its way to becoming resistant to antibiotics and becoming increasingly difficult to treat. This left three bacteria that the second unknown could be Staphylococcus aureus, Staphylococcus epidermidis, or Enterococcus faecalis. The milk agar was incubated at 37 degrees Celsius for 48 hours. coli can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses" (CDC). After successful isolation of both bacteria, there were no more issues encountered in identifying either. Professor Snaic advised to do another MSA agar from the original stock, but this time with a sterile swab instead of an inoculating loop. Selecting the correct antibiotic for this particular bacteria is especially important. In the medical field bacteria and infections of different kinds are the core of the practice. After incubation, this nutrient agar had great results with many isolated colonies. This test was also incubated at 37 degrees Celsius for 48 hours and returned a good isolated colony. aeruginosa is a facultative aerobic; its preferred metabolism is respiration." (MicrobeWiki) People that are most at risk for infection of P. Although some cases have been caused by swimming in pools or hot tubs with incorrect levels of chlorine. After observation, a sample was taken from the isolated colony on the streak plate and another streak plate was done with that, trying to further isolate the colonies. Even on an MSA agar, it took a few tries and several isolations to get the gram positive to successfully grow. This was the only test necessary to determine the unknown gram negative bacteria in unknown stock 123. This gave a positive result showing a hot pink broth, meaning the bacteria did produce urease. After confirming the gram negative bacteria, the process of isolating the gram positive bacteria began. A gram stain was done originally and found red rods identifying the bacteria as gram negative. All of the following tests were performed on this unknown: Procedure Purpose Reagents Results Unknown Lab Report: Unknown "C" Discussion/ResultsAfter a series of differential tests, a chart of bacteria was referenced to discover the unknown bacteria was E. As well as using the quadrant method to further isolate the colonies, a sample was taken from the best colony on the original streak and gram stained. The first unknown in #123 found to be a gram negative bacteria, was identified as Pseudomonas aeruginosa. "Pseudomonas Aeruginosa." - MicrobeWiki. The only problem that was encountered appeared when trying to isolate the gram positive bacteria. Materials and MethodsAn unknown bacterium labeled as letter "C" was handed out by Professor Woolam. A sample was taken from this colony and transferred to a nutrient broth agar to further isolate it. Upon reviewing the identification tables, the deciding biochemical test was the Casein test which tests for the production of the enzyme casease to break down the milk protein casein. Kenyon College, 30 June 2006. The Gram Stain resulted in a Gram-negative rod shaped bacteria which was not colonized. This MSA plate was incubated at 37 degrees Celsius for 48 hours. (2) This particular bacteria is so dangerous and pathogenic that it infects up to two thirds of critically ill patients in the hospital and is a leading pathogen in most medical centers with a mortality rate of 40-60% (2). Following the nitrate test was the Urea Test to determine if the bacteria produced urease. After observation there was a clear positive result, which showed the bacteria produced casease.

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