Determining unknown in microbiology lab

Determining an Unknown Through Deferential Stains and Biochemical Tests Introduction There are many reasons for knowing the identity of microorganisms. The reasons range from knowing the causative agent of a disease in a patient, so as to know how it can be treated, to knowing the correct microorganism to be used for making certain foods or antibiotics. This study was done by applying all of the methods that have been learned so far in the microbiology laboratory class for the identification of unknown bacteria. The identification process can be completed with a series of deferential stains and biochemical tests.

Creating a dichotomous key helps to limit the amount of biochemical tests done on an unknown organism and by observation and recording of data the unknown organism can be found. Materials and Methods The procedure for all tests performed was taken from Leboffe and Pierce’s Microbiology: Laboratory Theory and Application. The first and easiest data to record is the color of the bacterial colonies on a nutrient agar plate. Growing the cultures on the agar can be done by streaking a plate so as to isolate the bacterial colonies.

Then separate the two colonies that are different in growth on a TP plate by using the same streaking techniques with a sterile loop. Both bacteria will then undergo a simple stain and a gram stain. The simple stain uses the charged portion of a chromogen and allows it to act as a dye where it becomes positively charged as a result of picking up a hydrogen ion or losing a hydroxide ion. Thus, the cell becomes colored. The simple stain is useful for identifying the shape of the unknown bacteria. A gram stain is also performed on the unknowns.

The gram stain first uses crystal violet as the primary stain. Iodine is then used as a mordant fixodent to lock the stain in. Ethanol is used to decolorize the cell. Safarin is then used as the counter stain in order to show a contrast between the two colors. If the bacteria cell is gram negative, the decolorizer acts as a lipid solvent, causing the primary stain to leak out leaving the cell pink. If the cell is gram positive, the ethanol acts as a protein dehydrating agent causing the pores to close up and lock in the primary stain so the cell is purple.

Viewing these stained samples under a high power microscope lens enables the shape and gram stain to be determined. Streaking the gram negative bacteria on an EMB plate will help finalize the conclusion of the bacteria. Biochemical tests make use of enzymatic activities to differentiate among bacteria by products of biochemical reactions causing changes to the medium that have inoculated the organism with. This means that they identify whether specific reactions are occurring in the cell by testing for the presence or absence of enzymes or products.

Tests that show the presence of these enzymes or products are considered positive tests and the tests that show a lack of these enzymes or products are negative. The lactose fermentation test contains one of the following carbohydrates (sucrose, maltose, and lactose) and a pH indicator to detect acid formation. The broth tubes containing phenol red start as bright red by inoculating the medium with the gram positive colony it should indicate whether the bacteria is positive or negative. If fermentation is occurring and acid is being produced, the broth will change to a yellow color.

If no color change occurs, the result is a negative test. These broth tubes also contain a durham tube. This durham tube detects the presence of gas production from the fermentation reaction. If gas is produced, a bubble will form at the top of the durham tube. Urea is a product of decarboxylation of certain amino acids. The enzyme urease breaks urea down into NH3 and CO2. An orange broth containing urea is used for this test and needs to be inoculated with the gram negative bacteria. A pink color in the medium indicates a urease-positive organism, an orange or yellow is negative.

The IMViC test is a series of different tests that differentiate between enterics. One is the Indole test. This test tells whether the bacterium possesses tryptophanase which is the enzyme that breaks down tryptophan into indole. The agar contains tryptic soy broth so if the bacterium contains tryptophanase, indole is produced. This production of indole is seen by adding Kovac’s reagent which causes a red ring to be seen at the top of the tube. The citrate test is also used to see which kind of products the bacteria make.

It uses a green agar slant that contains sodium and ammonium phosphate. Bromythymol blue dye is late added as an indicator. Inoculation of the slant with a needle using a zig-zag then stab technique was used with the gram positive bacterium. Conversion of the medium to blue is a positive citrate result. All plates, slants, and broths were incubated at 37°C for 24? 48 hours. Results TestUnknown 16Unknown 16 Gram Stain+- ColorYellowYellow ShapeRodRod Lactosen/a+ Indolen/a- Ureasen/a+ Citrate++ Key: (+) = Positive Test (-) = Negative Test

N/A = Not Used for Determination Discussion The gram stain and bacteria shape were the most helpful in narrowing down the scope of possible unknown bacterium thereby narrowing down the number of biochemical tests needed to perform. The gram positive bacteria were yellow with rod shape. This narrowed the bacteria possibilities to only Bacillis subtillis and Bacillis cereus. In order to differentiate between B. subtillis and B. cereus the citrate test was performed. The conversion of the slant from green to blue indicates a positive citrate test result.

Thus, concluding that one of the bacterium is Bacillis subtillis; a rod shaped, gram negative, citrate positive organism. The shape of the gram negative unknown did not help in its identification. The gram stain and EMB plate were helpful in identifying that the bacterium was indeed a gram negative. After that a process of positive or negative results helped to eliminate all but one possible unknown. First test performed on the gram negative bacteria was the lactose test. The results of the PR lactose broth with durham tube indicated fermentation with acid and gas end products.

This narrows the bacteria prospects to Klebsiella pneumoniae, Escherichia coli, and Enterobacter aerogenes. The next test performed was the indole test which concluded the bacteria to have no reaction and be indole negative, narrowing down the bacteria to K. pneumoniae and E. Aerogenes. The final test perfomed being the urease test resulted in urease positive. That led to the conclusion that the unknown gram negative bacterium is Klebsiella pneumoniae. References Leboffe, Michael J. , and Burton E. Pierce. Microbiology: laboratory theory and application. Third ed. Englewood, Colo. : Morton Pub. Co. , 2010. Print.