

Identifying unknown bacteria | essay



**ASSIGN
BUSTER**

Introduction

The intent of this report is to identify Unknown #12. The conclusion of Unknown #12's identity was reached after a series of tests and subcultures were conducted using aseptic techniques. Aseptic techniques are necessary in order to avoid contamination of the culture being examined. Dr. Burne assisted in the conclusion of the identity of the unknown by informing the class of the results for the Oxidase and Lipase tests. And, while these two tests were not conducted by the students, they were still an important part of the identification process.

Procedures & Tests Conducted:

Subculture: The objective of this culture technique is to take a small portion of the Unknown bacterium using aseptic techniques, and transfer it into a tube of Nutrient Broth. This procedure must be repeated several times (almost daily) throughout the study of the bacteria in order to maintain a fresh and pure culture of the Unknown. If this procedure isn't repeated, the bacteria will eventually die from lack of nutrients and can give false positive results.

Conventional Gram Stain: This differential stain is conducted using aseptic techniques and is used to determine whether the unknown bacterium is Gram Negative or Positive. This stain can also help distinguish what shape the given bacterium is. In a clinical setting, determining whether bacteria are gram negative or positive, helps determine a quicker method of treatment. In relation to the stain itself though, gram negative and positive bacteria both stain purple initially when the crystal violet (primary stain) is applied. However, when iodine (a mordant) is applied, it combines with the crystal

violet to form a crystal violet-iodine (CV-I) complex. Once formed, “ this complex is larger than the crystal violet molecule that entered the cells and because of its size, it can’t be washed out.” (Tortora, 69) The CV-I is thus retained in the peptidoglycan layer of the gram negative and positive bacteria until Gram’s Acetone Alcohol (the decolorizer) is applied. The decolorizer eats through the thin layer of peptidoglycan in gram negative bacteria’s cell wall, allowing the CV-I to escape and causing the bacteria to become colorless. It’s for this reason it becomes necessary to apply a counterstain.

The counterstain applied in this procedure is Gram’s Safranin, a red/pink color dye. When applied though, the counterstain has no effect on the gram positive bacteria because the CV-I is retained in its cell wall due to its thicker layer of peptidoglycan in the cell wall. The counterstain is basically applied to give the gram negative bacteria some color. In the end, when examined under a microscope, gram negative bacteria will appear pink/red and gram positive will appear purple.

In terms of shape, when examined under a microscope, gram negative bacteria typically appear as straight rods, while round/cocci shaped bacteria are generally considered to be gram positive bacteria. However, there are a few cocci shaped gram negative bacteria as well. Also, it should also be kept in mind, that while shape is a characteristic of bacteria, some bacteria possess the ability to change shape; thus shape shouldn’t be strictly relied upon for identifying the unknown bacteria.

Streak Plate: This procedure, a culture technique, is performed using aseptic techniques and is used to obtain pure, isolated colonies. This procedure is usually performed before any biochemical tests “ in order to adequately study and characterize.... the bacterial species.” (Harley, 95) To obtain the isolated colonies, an inoculating loop is used to transfer the bacteria from a broth tube to a plate of nutrient agar or triptic soy agar. The inoculating loop is used to make about five individual lines, separate from one another. Once the lines are made, the plate’s incubated upside down and allowed to grow for 48 hours or so and then checked for bacterial growth. If the isolation plate is considered to be a success, the plate is kept for usage in the biochemical tests to be run on the bacterium. If the plate is considered of no use, the procedure is repeated until a successful plate is obtained.

Fermentation of Sugars Tests: This series of biochemical tests, preformed with aseptic techniques, is used to check “ the ability of microorganisms to ferment carbohydrates.” (Harley, 130) If the bacteria possess the ability to ferment the carbohydrate it’s tested against, the acid produced will lower the pH of the phenol red (pH indicator) and the liquid will turn yellow. To determine if the bacterium produces gas from the fermentation of the carbohydrates, an inverted (Durham tube) is used in the same tube (of sugar). If gas production occurs, bubbles will be present in the Durham tube. For the purposes of this test, the following sugars were tested: Glucose, Sucrose, Lactose, Maltose, Mannitol, Sorbitol, Xylose, Dulcitol, and Arabinose.

SIM test: This biochemical test, performed with aseptic techniques, is used to check for the production of Hydrogen Sulfide (H₂S) and Indole, as well as Motility. To begin this test, a portion of the bacteria must be transferred with <https://assignbuster.com/identifying-unknown-bacteria-essay/>

an inoculating needle from either a broth or solid culture to a tube of SIM agar. To do this, the needle is used to stab the media. The SIM agar, which “contains...ferrous ammonium sulfate, $Fe(NH_4)SO_4$, as an H_2S indicator,” (Harley, 151) will produce an “insoluble black ferrous sulfide precipitate that can be seen along the line of stab inoculation” (Harley, 151) if positive. If negative, there will be no black precipitate visible. In regard to motility, if the bacterium is positive, it will grow away from the stab line; if negative, growth will only be present in the stab line. A positive H_2S and motility test sometimes results in the entire tube turning black; if this is the case another motility test will be needed in order to determine the true motile state of the bacteria.

To check for the production of Indole, a substance called Kovac's reagent must be added to the top of inoculated tube. If a bright red color quickly results after adding about five drops of the Kovac's reagent, it indicates a positive result, while the lack of a reddish color, means a negative result. A positive result for the Indole test means that the bacterium contains the enzyme tryptophanase. The presence of this enzyme indicates that the bacteria “can hydrolyze tryptophan to its metabolic products, namely indole, pyruvic acid, and ammonia. The bacteria use pyruvic acid and ammonia to satisfy nutritional needs,” (Harley, 156) while Indole isn't used and is why “it accumulates in the medium” (Harley, 156) and reacts with the Kovac's reagent when applied.

Catalase Activity Test: This biochemical test is used to see if the bacterium “contains the enzyme superoxide dismutase, which catalyses the destruction of superoxide, and either catalase or peroxidase.” (Harley, 172) Essentially

<https://assignbuster.com/identifying-unknown-bacteria-essay/>

this enzyme's presence helps the bacterium to defend itself " against toxic O₂ products." (Harley, 172) To check for the enzymes presence or absence, hydrogen peroxide (H₂O₂) has to be added to a triptic slant that's been inoculated, using aseptic techniques with the unknown bacterium. Once the hydrogen peroxide's been added, bubbles will appear if positive, or no bubbles will appear if negative. Bubbles forming indicate that when the H₂O₂ was added to the medium, that it reacted with the superoxide dismutase enzyme and O₂ gas was released.

Gelatinase Activity Test: This biochemical test checks the bacteria's ability " to hydrolyze gelatin by secreting a proteolytic enzyme called Gelatinase." (Harley, 167) To inoculate the tube of nutrient gelatin, one must stab the media with an inoculating needle that contains a portion of the Unknown using aseptic techniques. If the bacterium is positive for Gelatinase activity, the media in the inoculated tube will no longer be in a solid state. The nutrient gelatin will become liquefied. The tube will remain this way even after being refrigerated for half an hour. However, if negative for Gelatinase activity, the nutrient gelatin that was inoculated will remain a solid. This test can also be used in a clinical setting to determine " the pathogenicity of certain bacteria...and ability of a bacterium to break down tissue collagen and spread throughout the body of a host." (Harley, 168)

EMB & ENDO Cultures: These two biochemical tests are used to check the bacteria's ability to ferment lactose. Both tests are performed with aseptic techniques. With the Eosin Methylene Blue (EMB) agar, this particular test is mostly intended to isolate and culture the bacteria but also show the bacteria's " response to the fermentation of lactose and/or sucrose by

microorganisms.” (Power, 220) If non-fermenting, the bacteria will be clear or colorless. If fermenting, the bacteria will have a “ characteristic green metallic sheen due to the rapid fermentation of lactose.” (Power 221) With the ENDO, when inoculated, if a blue-black color appears, it indicates that the bacterium is a lactose fermenting organism; however, like the EMB if no color change results, the bacteria is non lactose fermenting.

Phenylalanine Deaminase Test: This biochemical test checks the bacteria’s ability to remove “ amino group, NH_3^+ , from phenylalanine.” (Harley, 199) Once the tube has been inoculated using aseptic techniques, add ferric chloride to the plate of agar in trace of streak. If the test is positive, a green color will be visible. If negative, no color change will be observable. A positive result indicates that the bacterium “ produces the enzyme phenylalanine deaminase, which deaminates phenylalanine producing phenylpyruvic acid.” (Harley, 200) The ferric chloride (FeCl_3) in turn, combines with the phenylpyruvic acid and creates the green color observed.

Oxidase Test: This biochemical test, while using aseptic techniques, is performed in order to determine whether or not the given bacteria can “ produce cytochrome oxidase.” (Harley, 182) The test is performed by adding an oxidase test strip to colonies that have grown on a plate. If the strip becomes a dark purple, it’s positive. However, a light pink color or no change in color indicates a negative result. A positive result indicates that the bacteria undergo aerobic respiration because “ cytochrome oxidase uses O_2 as an electron acceptor...in the electron transport system.” (Harley, 182)

Second Motility Test: This biochemical test is strictly used to see if the bacteria's motile or not. To perform this test, a portion of the bacteria must be transferred from a broth culture to the SIM agar using aseptic techniques. In transferring the bacteria, the media has to be stabbed with an inoculating needle. After some time passes, if observation indicates growth away from the stab line, the test is positive, if there's no change or observation of growth, it's negative. This test is typically used to confirm the motile state of the bacteria observed in the SIM test.

Thioglycollate Test: This biochemical test is checking for the bacteria's oxygen requirements. For this test, the media used is Thioglycollate. Once the tube of Thioglycollate is inoculated using aseptic techniques, if the bacterium requires oxygen, it'll grow at the top of the inoculated tube. If it requires no oxygen, it'll grow at the bottom. However, if the bacteria's a "facultative anaerobe, it will grow either aerobically or in the absence of O₂." (Harley, 112)

Urease Activity Test: This biochemical test is used to figure out if the bacterium has the ability to "produce an enzyme called Urease that attacks nitrogen and carbon bond in ... urea." (Harley, 187) To detect the presence of the Urease enzyme, phenol red is used as a pH indicator. After inoculating a tube of Urea broth with the unknown, using aseptic techniques, if the test is positive, a dark pink or fuchsia color should be visible. However, if negative an orange color or something other than a pink color will be visible. The pink color in a positive result, occurs because of "ammonia accumulating in the medium and making it alkaline" (Harley, 188) once urea has been broken down.

IMVIC Test: This biochemical test, conducted using aseptic techniques, is composed of “ Indole, Methyl Red, Voges-Proskauer and Simmons-Citrate.” (Harley, 156) The Methyl Red (MR) part of the test is checking to see if the bacterium’s a mixed acid fermenter or a Butanediol fermenter. The Voges-Proskauer (VP) part of the test is looking to see if the bacterium ferments glucose. The Citrate portion of the test is trying to figure out if “ the bacteria can use Citrate as a sole carbon source for its energy needs.” (Harley, 157) With the MR test, if it’s positive, the red color will stay or intensify. If negative, it will lose the red color. The VP test is positive, if a color other than a watery yellow appears. The VP’s negative if no color change occurs. The Citrate test is positive if a deep blue color appears. If color remains green, it’s negative. On a side note, with the MR and VP tests, the VP test is only about 70% accurate, so if results for the MR and VP tests are negative-negative or positive-positive, only the MR test should be relied on, and the VP test should be assumed to be the opposite result of the MR.

Lipid Hydrolysis: This biochemical test is seeking to figure out if the bacteria can “ hydrolyze host cell phospholipids.” (Harley, 145) After using aseptic techniques to inoculate a tube, if the bacterium is lipase positive, a blue color should be present. If negative, a lavender color will remain. A positive result and the ability of the bacteria to use host cell phospholipids results in “ the release of fatty acids.... and can contaminate food products and cause spoilage.” (Harley, 146)

Nitrate Reduction: This biochemical test is checking the ability of the bacteria to reduce Nitrate to Nitrite. If the bacteria possess the enzyme Nitrate reductase, the test is positive and a red color will be observable in

the tube that was inoculated using aseptic techniques. If the test result is negative, there will be no color change. However, there's a slight glitch in this test and if negative, a nitrate reagent must be added to the medium, to check the result for confirmation. The nitrate reagent is a mix of zinc dust and water. Once added to the tube of nitrate, if a red color results after adding the reagent, then it's actually a negative test result. If still negative, the actual reaction is positive, but the bacteria kept going after degrading nitrate to nitrite and ended up producing ammonia.

Antibiotic Sensitivity: This test is simply used to check the ability of the bacteria to grow on or around certain antibiotics. The bacterium is spread as a lawn on a plate of Múeller-Hinton agar and the discs of antibiotics are dropped into the plate in separate locations. If the antibiotics are successful in preventing or eliminating growth of the bacteria, there will be a clear space around the disc of the antibiotic. Otherwise, growth on or around the antibiotic indicates it's not useful in combating growth of the bacteria. The antibiotics tested against Unknown #12 were Penicillin, Kanamycin, Bacitracin, Doxycycline and Vancomycin.

Antiseptic Sensitivity: This test is similar to the antibiotic test, except it is checking the ability of the bacteria to grow on or around antiseptics. With this test, the bacterium is spread as a lawn on Múeller-Hinton agar in a plate and paper discs with the antiseptics on them are dropped in separate places around the plate. If the bacterium's able to grow in or around the disc containing the antiseptic, it indicates that the antiseptic is of no use in preventing growth of the bacteria. However, if the antiseptic has no growth around it, it's successful in inhibiting growth of the bacteria. For this test, the <https://assignbuster.com/identifying-unknown-bacteria-essay/>

antiseptics tested were Listerine (advanced) mouthwash, Hydrogen Peroxide, Germ X, Complete Easy Rub Contact Solution and Equate Antibiotic Hand Soap.

Results of Tests Conducted:

The table below represents the result of each technique and biochemical test conducted.

Test Performed:

Results & Comments:

- Gram Stain

Gram Negative (Cocci, bacteria are round in appearance and in chains, but could be groups of straight rods as well with a slight curved appearance)

- Glucose Fermentation

Positive for Acid; Positive for Gas Production

- Sucrose Fermentation

Positive for Acid; Positive for Gas Production

- Lactose Fermentation

Positive for Acid; Positive for Gas Production

- Maltose Fermentation

Positive for Acid; Positive for Gas Production

- Mannitol Fermentation

Positive for Acid; Positive for Gas Production

- Dulcitol Fermentation

Negative for Acid; Negative for Gas Production

- Xylose Fermentation

Positive for Acid; Positive for Gas Production

- Sorbitol Fermentation

Positive for Acid; Positive for Gas Production

- Arabinose Fermentation

Positive for Acid; Positive for Gas Production

- SIM Test

H₂S: Positive, black precipitant visible

Indole: Negative, didn't turn red with Kovac's reagent

Motile: Positive, growth throughout tube

- Catalase Activity

Positive, bubbles formed when hydrogen peroxide was added

- Gelatinase Activity

Negative, still in solid state

- EMB & ENDO Cultures

EMB: Clear & Colorless in trace of Streak.

ENDO: Clear & Colorless in trace of Streak

- Phenylalanine Deaminase Test

Positive; turned green when citric acid was added in trace of streak

- Oxidase Test

Negative (Dr. Burne Said all unknown's were Negative for Oxidase)

- Second Motility Test

Positive, growth away from stab line

- Thioglycollate Test

Facultative Aerobe. The bacteria grew at the top, middle and bottom of the tube, but primarily at the top.

- Urease Activity Test

Positive, bright pink color visible

- IMVIC Test

MR: Positive, Red-Orange Color

VP: Negative, stayed yellow

Citrate: Positive, turned blue

- Lipid Hydrolysis

Negative (Dr. Burne told the class which were negative & positive).

- Nitrate Reduction

Positive, however zinc dust with water had to be added to the medium due to the fact the bacteria had gone too far and produced ammonia.

- Antibiotic Sensitivity

The only successful antibiotic in preventing growth of the Unknown was Kanamycin

- Antiseptic Sensitivity

The only successful antiseptics in combating growth of the Unknown were Hydrogen Peroxide, Equate Antibacterial Hand Soap and Listerine (Advanced) Mouthwash.

Conclusion:

Based upon the outcome of the above tests and procedures that were conducted, I believe Unknown #12's identity is *Citrobacter freundii*. This conclusion is based upon the fact that the Order, Enterobacteriales, are "facultatively anaerobic, gram negative rods that are motile-morphologically, the rods are straight and most are active fermenters of glucose and other carbohydrates." (Tortora, 309) This conclusion was further backed up by the result of the Oxidase test as "all members of the family Enterobacteriaceae are oxidase-negative." (Tortora, 285) The Enterobacteriales are the only Order that fits and concurs with the results of the tests performed and thus is why it's assumed to be the Order.

After determining the Order, I knew the bacteria belonged to the Gammaproteobacteria Class because of table 11. 1 in Microbiology: an Introduction. The bacterium's Family was narrowed down by the result of the Lactose, sugar fermentation test. Tortora states that "Escherichia, Enterobacter and Citrobacter, which ferment lactose to produce acid and gas, can be distinguished from Salmonella and Shigella, which do not." (285) Escherichia, and Enterobacter were both eliminated and Citrobacter

assumed as the family after the Citrate, Urea and SIM tests were complete. Citrobacter is the only family of the three that has a positive Citrate, H₂S, and Urea test. Citrobacter is also the only one of the three families that has a negative Indole test result most of the time.

Furthermore, the determination of the bacteria's Genus was able to be concluded after carefully examining the results of the other biochemical tests. Citrobacter freundii, according to Bergey's Manual has "no liquefaction by any strains," (Breed, 339) and has a positive Catalase test result. These results and conclusion were further reinforced by the outcome of the Antibiotic Sensitivity Test. Kanamycin, which was successful in preventing growth of the bacteria is "a bactericidal antibiotic which acts by inhibiting the synthesis of protein in susceptible microorganisms. Kanamycin sulfate is active in vitro against many strains of Staphylococcus aureus...and Citrobacter freundii and Citrobacter species... that are frequently resistant to other antibiotics." (Medpedia)

After reading the description of Citrobacter freundii, the findings are inconclusive with each other on the Gram Stain, most of the Fermentation of Sugars tests, Gelatinase, Indole, IMVIC, Citrate, SIM, Urea, Nitrate and Catalase tests. In addition, the results of each biochemical test and procedure conducted are assumed accurate, as there was no contamination of the culture or test materials used, due to aseptic techniques being exercised with each test and procedure performed.