Identification of unknown bacteria

Unknown Lab Report

- Haridja Kabuyi Ilunga

1. Abstract

To discover the identity of the unknowns that have been given to each student, a series of biochemical tests were requisite. The different biochemical tests that were perform was Methyl Red test, Vogues-Poskauer test, Citrate test, Urea Hydrolysis, Gelatin Hydrolysis test, Sulfure Indole Motility test and Triple Sugar Iron Agar slant. Prior to the performance of those tests, it was important to isolate the unknown in a Tryptic Soy Agar plate by doing the T-streak method and confirm with a Gram Stain that the unknowns were gram negative bacteria. Why was this step important? Because the six bacteria listed, *Escherichia coli* , *Enterobacter aerogenes* , *Klebsiella pneumoniae* , *Proteus mirabilis* , *Pseudomonas aeruginosa* and *Salmonella typhimurium* , were gram negative bacteria.

2. Introduction

Gram positive is the name given to bacteria that turn purple-violet after the Gram Stain. The cell wall of gram positive bacteria contain a thick peptidoglycan layer and perpendicular to that layer is a group of molecule called Teichoic acid which is unique to the gram positive. (Todar, kennet). They practically lack an outer membrane. The structure of their cell wall, they have high amount of peptidoglycan, enable them to retain the crystal violet stain during the gram stain (livre de lab).

Gram negative is the name given to bacteria that turn pink after the Gram Stain. The cell wall of gram negative is composed of a single layer of

peptidoglycan surrounded by the outer membrane (Todar, kennet). The outer membrane is contained Lipopolysaccharide or endotoxin; this component is toxic to animals. (le livre de lab) This is the reason of the pathogenicity of certain gram negative bacteria. During the Gram Stain, the lipid content is target by the alcohol, decolorizer, and make the outer membrane porous that unable the cell to retain crystal violet, the primary stain. (le livre de lab) They appear pink because they are taking the color of the counterstain, Safranin.

A gram stain was performed at the beginning of the experiment. A gram stain is the most common differential stain. It uses to differentiate cells by their color under the microscope; either they are gram negative (pink) or gram positive (purple violet). The gram stain uses four chemicals; Crystal violet (primary stain), Gram’s Iodine (mordant), 95% Ethanol (decolorizer) and Safranin (counterstain). The importance of this test was to confirm that the unknown given was a gram negative.

The Methyl Red test was performed to detect the production of mixed acid from Glucose fermentation. The Methyl Red test and the Vogue Proskauer test use the same broth solution that contain peptone, glucose and phosphate buffer. The composition of this solution is important. Each component has his role; the peptone provides protein, glucose provides a fermentable carbohydrate and the potassium phosphate resists pH changes. The organisms that product mixed acid fermentation overcome the buffer and are stable. The Methyl red test is a PH indicator; it is red when the pH is 4. 4, yellow when it is 6. 2 and orange is an inconclusive result. The indicator

for this test is Methyl Red. (Methyl Red (MR) test: Principle, procedure and results January 24, 2014 Tankeshwar Acharya)

The Vogues-Proskauer was performed to detect the organism production of 2, 3 Butanediol from glucose fermentation. It has the same broth solution as the Methyl Red test but it is uses for a total different purpose. The detection of 2, 3 Butanediol is not directly detected. Instead his precursor, Acetoin, is. Therefore the Vogues-Proskauer test tests for the presence of Acetoin. Its reagents are Barrit reagent A (alpha-napthol) and Barrits reagent B (potassium hydroxide). Barrit reagent A and B react with the glucose when it is broken down and form a red color which indicates a positive result. No color change is a negative result. (sur le net et le livre)

The citrate test was executed to determine the Sole Carbon Requirement. Citrate test is a nutrient utilization medium. It means that it is a highly defined media that differentiate organisms based on their ability to grow when an essential nutrient is strictly limited. This medium contains sodium citrate that serves as the sole carbon source and ammonium phosphate as the sole nitrogen source. The organisms that can utilize citrate as a sole source of carbon produce the enzyme citrate-permease which transport citrate into the cell and produce pyruvate from it. Pyruvate is utilized for further biochemical reactions. It also convert ammonium phosphate into NH3+NH4OH causing the media to become more alkaline. The indicator of the test is the Bromothymol blue dye which is green at pH6. 9 and blue at pH7. 6 and above. Therefore a positive result is a blue color and the negative result is a no color change. (Citrate utilization test: Principle, Procedure,

expected results and positive organismsMAY 17, 2013 TANKESHWAR ACHARYA et le livre).

A Urea hydrolysis test was executed to detect the production of the intracellular enzyme, urease. The role of the intracellular enzyme Urease is to hydrolyze Urea into ammonia and carbon dioxide. The broth of urea is a buffered solution of yeast extract, urea, peptone, potassium phosphate buffer and phenol red. Phenol Red is the pH indicator which turns yellow when the pH less than 6. 8 and pink when the pH greater than 8. 4. A positive result for this test is the pink change in color. If urea can be used by the microbe, alkaline breakdown products appear and the pH of the medium rises.

Gelatin Hydrolysis test was performed to detect the production of the enzyme Gelatinase. Nutrient gelatin is a differential medium that tests the ability of an organism to produce an exoenzyme, called gelatinase. This enzyme allows the organisms that produce it to break down gelatin into smaller polypeptides, peptides, and amino acids that can cross the cell membrane and be utilized by the organism. When gelatin is broken down, it can no longer solidify. If an organism can break down gelatin, the areas where the organism has grown will remain liquid even if the gelatin is refrigerated. Liquid is a positive result for gelatinase and solid is a negative result for gelatinase. (le livre).

SIM test was performed to detect the reduction of sulfur, the production of indole from tryptophan and the motility. SIM medium is a semi-solid media that tests three different parameters which are Sulfur Reduction, Indole

Production and Motility. it contains an iron-containing compound, casein and animal tissue as sources for amino acids and sulfur in the form of sodium thiosulfate. Two enzymes can be used to reduce sulfur to hydrogen sulfide and produce gas, those are cysteine desulfurase and thiosulfate reductase. If an organism can reduce sulfur to hydrogen sulfide, the hydrogen sulfide will combine with the iron to form ferric sulfide. If there is any blackening of the medium, it indicates the reduction of sulfur and is a positive result. The Kovac's reagent that you add to the SIM medium test for indole contains hydrochloric acid, p-dimethylaminobenzaldehyde (DMABA), and n-amyl alcohol. DMABA reacts with indole to produce a red color. It is a positive test for indole.

TSIA test was performed to detect the production of gas, sugar utilization and sulfur reduction. Slide 25-27. Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate organisms that have the ability to reduce sulfur and ferment carbohydrates. If an organism can ferment any of the three sugars present in the medium, the medium will turn yellow. The reaction that produced acid reverts in the aerobic areas of the slant, and the medium in those areas turns red, indicating alkaline conditions. The anaerobic areas of the slant, such as the butt, will not revert to an alkaline state, and they will remain yellow. If an organism can reduce sulfur, the hydrogen sulfide gas which is produced will react with the iron to form iron sulfide, which appears as a black precipitate. If the butt of the slant is obscured by the precipitate, look at the top of the slant to determine if the organism could ferment only

dextrose (red), or if it could ferment either lactose and/or sucrose (yellow). If the fermentation produced gas, you may see fissures in the medium, or the entire slant may be raised above the bottom of the test tube.

3. Materials and Methods

Unknowns were given to each student to find their identities with the use of different biochemical tests. The unknowns were in a broth. A T-streak test was executed in a TSA plate to isolate the pure colony. An extract TSA broth was also provided to streak and keep as back up. A gram stain was performed to confirm that the bacterium was a gram negative. After 24h of incubation in the hot room at 37Ëš, the bacteria have grown and were ready for the biochemical tests. General inoculating and aseptic protocols and techniques were followed.

The TSIA slant was heavily inoculated. This means that the medium was stab to the bottom of the media with an inoculating needle. The top of the slant was also streak. This technique provided both aerobic and anaerobic growth environments for the microorganism. The incubation time was 48h in the hot room at 37Ëš.

The SIM tube was inoculate using an inoculating needle. The media was stab until reaching the 2/3 of the media and reformed carefully following the original stab line. This step was important for the detection of the motility. The incubation time was 24h in the hot room at 37Ëš. After the incubation, 3mm of kovack reagent was added to the tube. The results were observed.

Citrate slant was inoculated using inoculating needle. It was a light inoculation which means that only the top of the slant was streak. The time of incubation was 24h in the hot room at 37Ëš. The results were observed.

The urea broth was incolated with the unknown using an inoculating loop. The time of incubation was 24h in the hot room at 37Ëš. Then the results were observed.

The Gelatin tube was heavily inoculated. It was stab once until the inoculating needle reach the bottom of the tube and remove following the original stab line. The time of incubation was 8 days in the hot room at 37Ëš. After the incubation time, the gelatin stayed at room temperature for 1h and then the results were observed.

The methyl red broth was inoculated using an inoculating loop. The time of incubation was 24h in the hot room at 37Ëš. After the incubation time, 15 drops of methyl red were added to the broth and the results were observed.

The Voges-Proskauer broth was inoculated using an inoculating loop. The time of incubation was 3 days in the hot room at 37Ëš. After the incubation time, 15 drops of Barrits reagent A and 5 drops of Barrits reagent B were added to the broth. After 1h in room temperature, the results were observed.

4. Results

After the gram stain, the unknown was observed under oil immersion objective which has a total magnification of x1000. The bacterium was gram negative which has a rod shape.

After the inoculation and the 24h incubation of the TSIA slant, the result was positive for gas. The entire slant was raised above the bottom of the test tube. This means that the fermentation produces gas. After another 24h of incubation, the top of the slant turn red. This means that the TSIA slant was positive for glucose. The organism could ferment only glucose. It also means that the bacterium was pathogenic. There was no black precipitate in the media which means that the organism cannot reduce sulfur.

After the incubation time, the kovack reagent was added to the SIM semi-solid media. There was no change in the media except a small turbidity on the bottom of the media. This means that the test was weakly positive for motility but negative for sulfur reduction and indole production. DMABA did not react with indole to produce a red color.

After the incubation time, the citrate turned blue which is a positive result for the production of the enzyme citrate-permease. The organism also converts ammonium phosphate into NH3+NH4OH causing the media to become more alkaline. The Bromothymol blue dye (the indicator) indicates by the color blue that the pH was at 7. 6 or above.

After the incubation time, the urea test was pink which indicates a positive result. This indicate the production of the intracellular enzyme, urease and that the pH of the solution was 8. 6.

After the incubation time, the reagent Barrits A and B was added. But the Voges-Proskauer did not change in color. This was a negative for the production of acetoin therefore the organism doesn’t produce 2, 3 Butanediol. Barrit reagent A and B did not react with the glucose.

After the incubation time, methyl red was added to the broth. The methyl red test was inconclusive. This means that it had an orange color. An orange color indicates that the organism has a pH between 4. 0 and 6. 0. It does indicate the production of mixed acid.

After the incubation time, the gelatin slant stayed 1h in room temperature. The result was negative for the enzyme gelatinase. This enzyme allows the organisms that produce it to break down gelatin into smaller polypeptides, peptides, and amino acids that can cross the cell membrane and be utilized by the organism. But in this case, the gelatin was not broken down, it was solidify.

5. Discussion

A series of biochemical tests were conducted in order to identify the unknown 20. Then the results were compared with the chart of known characteristics. After having collected all the data, the list of bacteria was reduced to only two choices; the *Proteus pneumonia* and the *Klebsiella pneumoniae .* The SIM test, the MR-VP, the citrate test and the urea test did not help much. They all have the same result in both organisms. There were no change in media color after adding the kovak reagent; The sulfur reduction was negative, the indole production was negative; DMABA did not react with indole to produce a red color. the motility was very weak. The MR was inconclusive, after adding methyl red, the color became orange which can indicate a small production of mixed acid. there was no production of acetoin, the VP test was negative. There was the presence of citrate permease, they can utilize citrate as a sole source of carbon and produce the

enzyme citrate-permease which transport citrate into the cell and produce pyruvate from it. It also convert ammonium phosphate into NH3+NH4OH causing the media to become more alkaline. The Bromothymol blue changes in blue to indicate that the pH is 7. 6 or above. There was the production of urease. The role of the intracellular enzyme Urease is to hydrolyze Urea into ammonia and carbon dioxide. Phenol Red (the pH indicator) turned pink which means that the pH is greater than 8. 4. the tests that were helpful to identify the unknown was the TSIA test and the Gelatin test. The TSIA test showed the production of gas and glucose fermentation. The top of the slant turn red after 48h of incubation and the bottom of the slant went up after only 24h of incubation which show that the fermentation of the sugar produces gas. The gelatin was solid after 1h in room temperature that indicates the absence of the enzyme gelatinase. This two test were really helpful because they were opposite in the two bacteria.

After all those test the unknown 20 was identify as *Klebsiella pneumoniae*