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BIO 3302L - E321

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What is it? Unknown #20

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Introduction

Microbiology, the branch of science that studies microorganisms. I really did not understand the importance of that the very first time I heard it. I have since then come to understand. What is a microorganism? A microorganism (or microbe) essentially is a unicellular or multicellular microscopic organism, including bacteria, viruses and fungi. Microorganisms play an important role in our everyday lives. It is very important to be able to identify and study them. There are good microorganisms out there, but there are also plenty of bad ones which are called pathogens. Pathogens cause a great amount of harm to humans, animals and plants. Being able to identify pathogens enables the prevention and treatment of them. This can greatly help to improve the lives of us humans [1]. For example, antibiotics could not be created without the identification of harmful microorganisms. We also learn how these many different pathogens affect the human body, as well as the body's response to these invaders. Being able to identify microorganisms also helps in maintaining the safety of our food supply. There are many instances in which we hear that meat or greens has been recalled due to contamination. Being able to identify these contaminants can help in preventing them from infecting our food supply. Another reason is to learn more about human microbiota.[2] Human microbiota are the microorganisms that live on and in humans. Something very important that must be done during the study of microbiology is the aseptic technique. The aseptic technique means following all procedures to eliminate the chance of contamination [3]. For this lab practical, each student was given two unknown bacteria in a liquid broth. Each tube of "unknown" contained a gram positive and a gram negative

bacteria. Using aseptic techniques, we had to prepare various cultures and perform various biochemical tests to determine which bacteria we were given. I was given unknown #20. Due to the many steps and tests involved, this was done over a period of three days.

Materials and Methods

Figure 1 shows Unknown #20 in a liquid broth with a cloudy appearance. The cloudy appearance means there is growth in the tube.

The first step was to prepare a pure culture on various types of media. The media used were Blood Agar, MacConkey, Phenylethyl Alcohol (PEA) and Mannitol Salt.

Blood Agar

A differential medium that determines hemolytic patterns. The expected observations are a brown or green color which indicate alpha hemolysis, a halo effect indicating beta hemolysis, or no change indicating no hemolyzation.

MacConkey

Both a selective and differential medium. It isolates and differentiates enterics based on the ability to ferment lactose. Gram negative bacteria that ferment lactose will produce acid and the colonies will appear red or pink.

Phenylethyl Alcohol (PEA)

A selective medium that cultivates gram positive bacteria while inhibiting gram negative bacteria by disrupting the DNA. Thus, the bacteria will either show growth or no growth.

Mannitol Salt

Both a selective and differential medium containing a high concentration of salt. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow. Mannitol salt is a key medium used when identifying bacteria from the genus *Staphylococcus*.

After creation of the pure cultures was complete, they were left to incubate overnight. Once the incubation period was completed, two Gram stains were prepared. The first was done using the culture from the PEA and the second using the culture from the MacConkey.

The next step was to inoculate the unknown into various media for biochemical testing. Biochemical testing involves identifying the presence and/or activities of enzymes. This aids in the identification of unknown bacteria.

Cultures from the PEA and MacConkey plates were inoculated onto a neutral agar plate. This would be used to conduct the oxidase and catalase tests. Cultures from the PEA and MacConkey plates were also inoculated onto various tubes which will be discussed later in this section.

Oxidase Test

The oxidase test is used to detect the presence of cytochrome-c. Bacteria that are oxidase positive are aerobic and use oxygen as an electron acceptor during respiration.

To perform the oxidase test, a drop of oxidase reagent is placed directly onto the bacteria in the neutral agar plate. If the area where the drop was placed turns blue, the test is positive for oxidase. If there is no change, the test is negative for oxidase.

Catalase Test

The catalase test is used to differentiate those bacteria that produces an enzyme catalase from non-catalase producing bacteria. It is key in distinguishing *Staphylococcus* from *Streptococcus*. A few drops of 3% hydrogen peroxide is placed directly on the bacteria in the neutral agar plate. If the area where the drops were places begins to bubble, the test is positive for catalase. If there is no change, the test is negative for catalase.

The other biochemical tests that were conducted were as follows: Triple Sugar Iron Agar (TSI), Urease activity, Bile Esculin, Nitrogen Metabolism, the multiple test system referred to as IMViC was designed to distinguish among members of the family *Enterobacteriaceae*. IMViC refers to Indole, Methylred, Voges-Proskauer (VP), and Simmons Citrate, and the Sulfide Indole Motility test (SIM). The Indole and SIM tests are done in the same tube. The Methylred and Voges-Proskauer test are done in the same tube but divided after incubation.

Triple Sugar Iron

TSI is used to differentiate gram-negative enterics due to carbohydrate fermentation and the production of hydrogen sulfide. TSI contains three sugars as its name states, the sugars are sucrose, lactose and glucose. Phenol red detects fermentation by turning yellow. There is also ferrous ammonium for detected hydrogen sulfide which will show

as black. Any signs of bubble formation would indicate gas production. The slant is inoculated and incubated, then results are observed. The possible results are listed on Table 1 below:

Table 1: Possible Results for Triple Sugar Iron Agar

Slant/Butt color	**Gas Produced**	**Interpretation**
No change/No change	no	No fermentation
Red/Yellow	no	Glucose fermentation
Red/Yellow	yes	Glucose fermentation and gas production
Red/Yellow plus Black precipitate	no	Glucose fermentation, Hydrogen sulfide produced
Yellow/Yellow	no	Glucose plus lactose and/or sucrose fermentation
Yellow/Yellow	yes	Glucose plus lactose and/or sucrose fermentation and gas production
Yellow/Yellow plus Black precipitate	no	Glucose plus lactose and/or sucrose fermentation, Hydrogen sulfide produced
Red/Red	no	No fermentation, Protein catabolism

Urease activity

This is a differential media that contains urea and phenol red. Certain bacteria can produce an exoenzyme called Urease which can split urea into ammonia and carbon dioxide. This helps to differentiate Urease-positive species of *Enterobacteriaceae.* The liquid broth is inoculated and incubated then results are recorded. The possible results are:

- No change = Negative for Urease
- Red/orange color = Positive for Urease, slow metabolism of urea
- Bright pink color = Positive for Urease, rapid metabolism of urea

Bile Esculin

Bile Esculin is a selective and differential media which is used to identify members of the *Enterococcus* genus. The bile salts inhibit the growth of other organisms. *Enterococcus* are able to hydrolyze esculin creating a blackening appearance. The slant is inoculated, incubated and results are then recorded. The possible results are:

- No change = negative
- Dark brown to black = positive

Nitrogen Reduction

This test is used to test if an organism can reduce nitrate to nitrite using the enzyme nitrate reductase. The liquid broth is inoculated and incubated. Next you must add two drops of sulfanilic acid and shake, then add two drops of dimethyl-alpha-naphthylamine and shake. Observe any color change. If the broth turns red, the results are positive. If there is no change you must add a very small amount of powdered zinc, shake and repeat steps one and two. If the broth turns red now, the results are negative.

Sulfide Indole Motility

SIM is three tests in one. During inoculation the semi-solid media is stabbed.

The presence of hydrogen sulfide is noted if after inoculation and incubation there is a blackening of the media.

The indole test is used to determine if a bacteria can convert trytophan into indole. After inoculation and incubation, five drops of Kovac's reagent is added. If there is a formation of a red ring on the top layer of the broth, the test is positive for indole. If there is no change, it is negative.

Motility is noted if there is diffuse growth from the inoculated site after incubation.

Methylred and Voges-Proskauer

After inoculation and incubation, half of the liquid broth is poured into a clean tube in order to run the tests separately.

Methylred is used to distinguish facultative anaerobic enterics. The bacteria must first be able to metabolize glucose then ferment it. After inoculation and incubation, five drops of Methyl red is added. If the broth turns a pink or cherry red, the test is positive. If there is no change, it is negative.

Voges-Proskauer is performed to detect acetoin. It is key in differentiating *E.coli* from *E. aerogenes* and *Klebsiella*. After inoculation and incubation, five drops each of the reagents VPA which is Alpha-naphitol and VPB which is potassium hydroxide are added to the tube. If the broth turns a pink or cherry red, the test is positive. If there is no change, it is negative.

It is important to note that the results of these two tests are usually the opposite.

Simmons Citrate Test

This test determines the bacteria's ability to utilize citrate as its only source of carbon. A positive result indicates alkaline conditions. The slant is inoculated lightly without stabbing the media, then incubated. If the slant turns blue the test is positive. If there is no change, it is negative.

Results

Cultures

The table below shows the results of the pure cultures:

Table 2: Results of Pure Cultures

Medium	**Observation**	**Results**
Blood Agar	brown	Alpha hemolysis
MacConkey	growth, pink	Gram negative, lactose fermenter
PEA	growth	Gram positive
Mannitol Salt	growth, yellow	Tolerant of high salt, mannitol fermenter

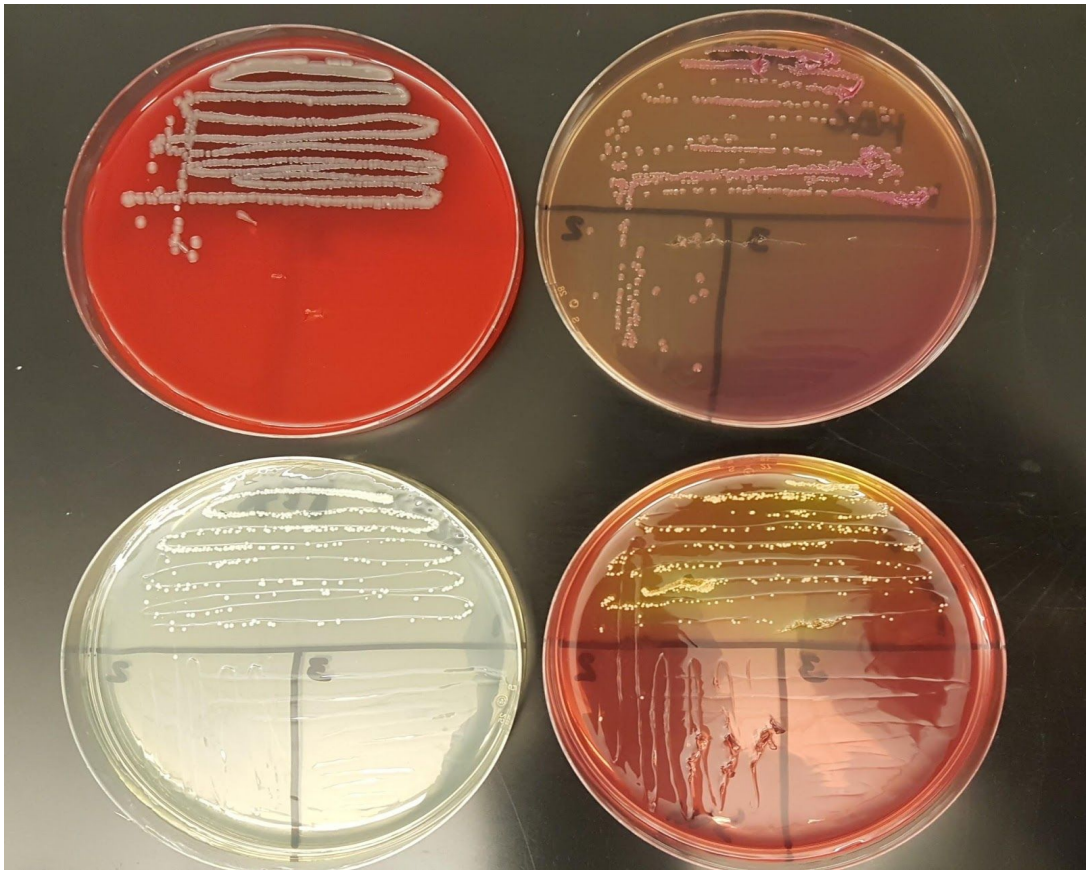

Figure 2 shows the results of the pure cultures that was listed in Table 2.

Gram Stains

The gram stain prepared from the PEA culture revealed purple cocci in clusters. The results show the bacteria is gram positive.

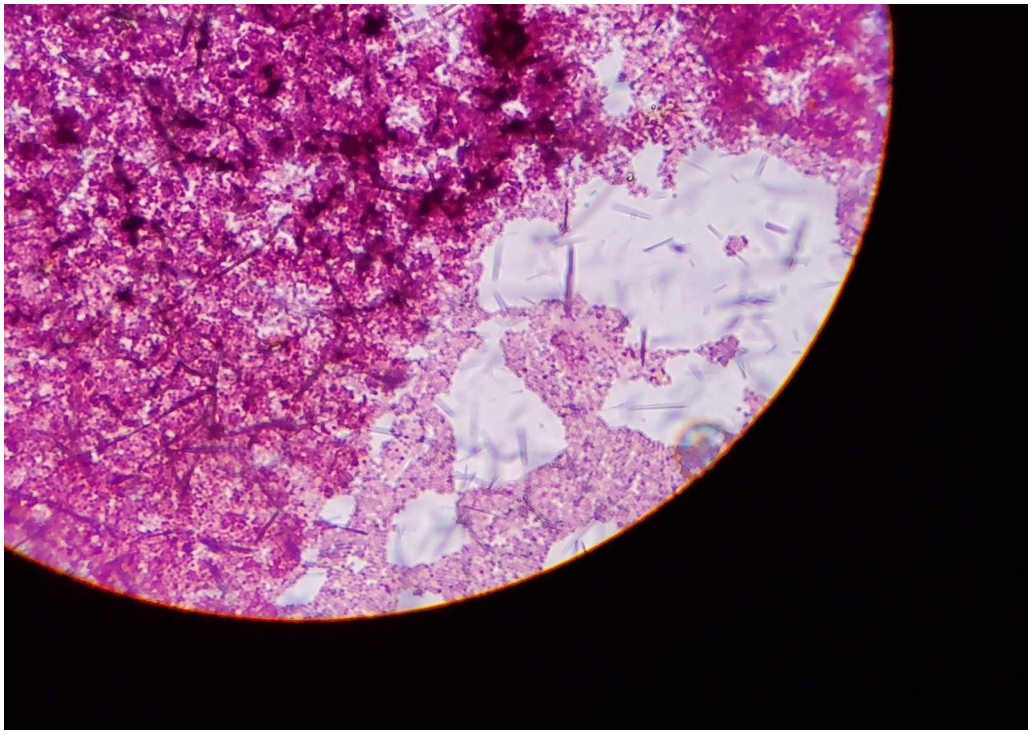

Figure 3: Results of gram staining show purple cocci.

The gram stain prepared from the MacConkey revealed small pink bacillus in clusters.

This indicates it is gram negative.

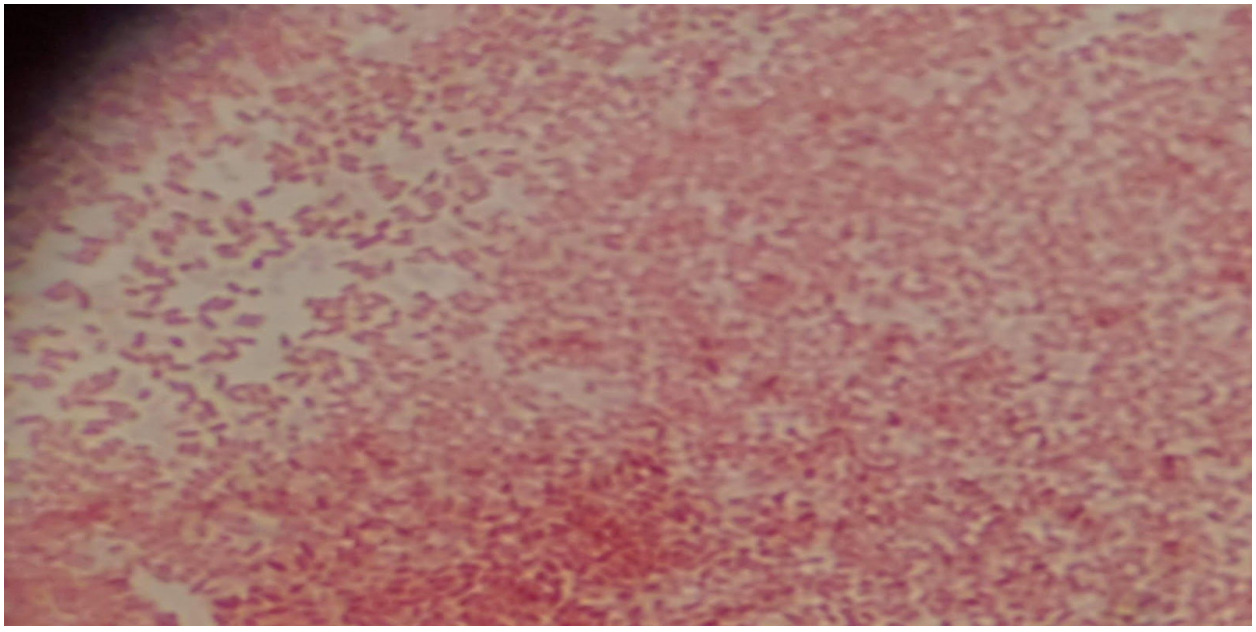

Figure 4: Results of gram staining show pink bacillus.

Oxidase and Catalase

The MacConkey media turned blue which indicates it is positive for oxidase.

The PEA media had no change, indicating it is negative for oxidase.

Both the MacConkey media and PEA began to bubble which indicates they are both positive for catalase.

TSI

Both the MacConkey and PEA tubes showed yellow slants and yellow butts and showed gas formation. This is indicative of glucose plus lactose and/or sucrose fermentation plus gas production. It is interesting to note that the MacConkey tube exhibited a larger amount of gas formation.

Urease

Both the MacConkey and PEA tubes turned a faint rose pink. This indicates that they are positive for Urease and have slow metabolism of urea.

Bile Esculin

The MacConkey tube turned black, indicating it is positive. The PEA tube was light brown indicating a negative result.

Nitrogen Reduction

The MacConkey tube showed no change after adding the reagents. Zinc was added and the reagents were added again and the tube turned orange, indicative of a negative result.

The PEA tube turned a brightish orange color after adding the reagents. This shows a positive result.

Sulfide Indole Motility

Both the MacConkey and PEA tubes were not black showing there is no production of hydrogen sulfide.

Five drops of Kovac's reagent was added to each tube to test for indole. Both had no change indicating they are negative for indole.

The MacConkey tube did show some turbidity indicating there is motility.

The PEA tube remained clear and the stab line was visible, indicating it is non-motile.

Methylred and Voges-Proskauer

After adding five drops of methyl red, the MacConkey tube turned a light red indicating a positive result. The PEA tube remained yellow indicating a negative result.

After adding five drops each of VPA and VPB, the tubes remained yellow, showing they are both negative.

Simmons Citrate

The MacConkey tube turned blue indicative of a positive result and the PEA tube remained green indicating a negative result.

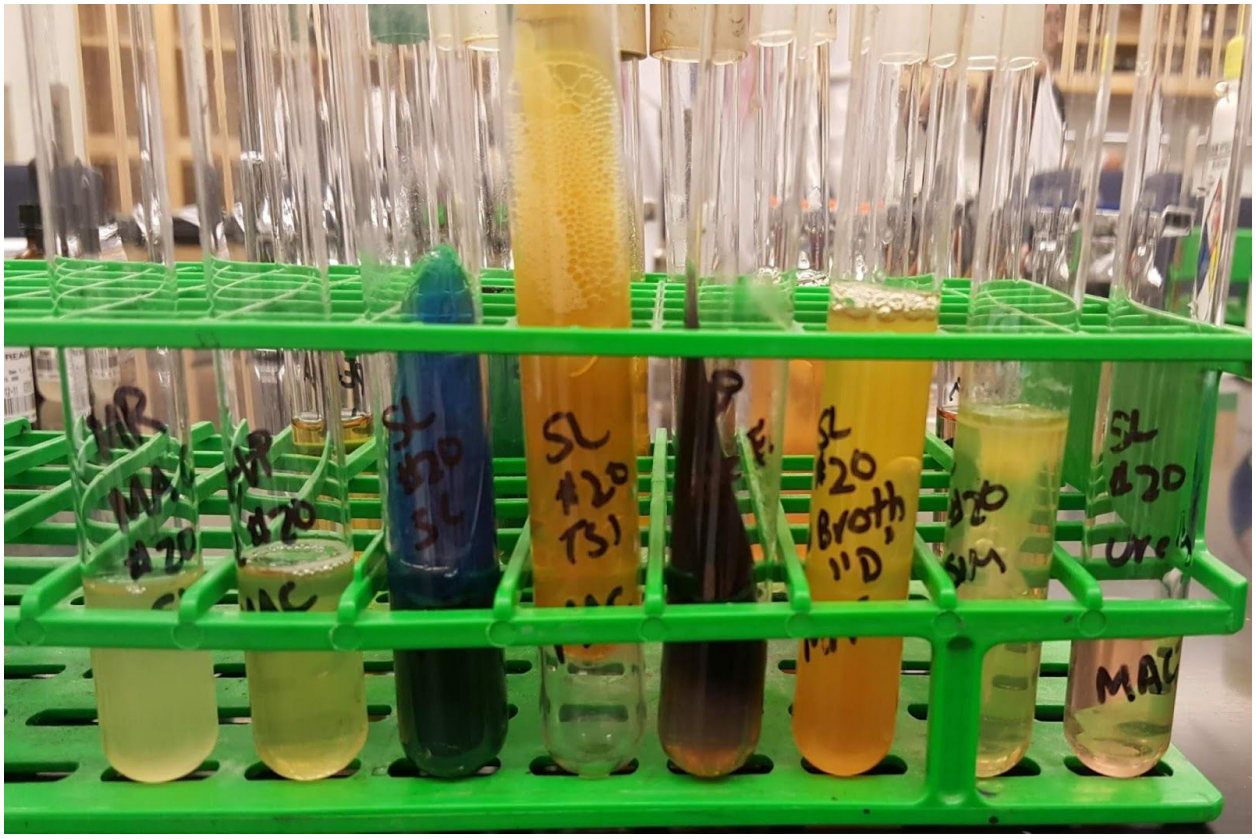

Figure 5 shows the results stated above for the MacConkey culture.

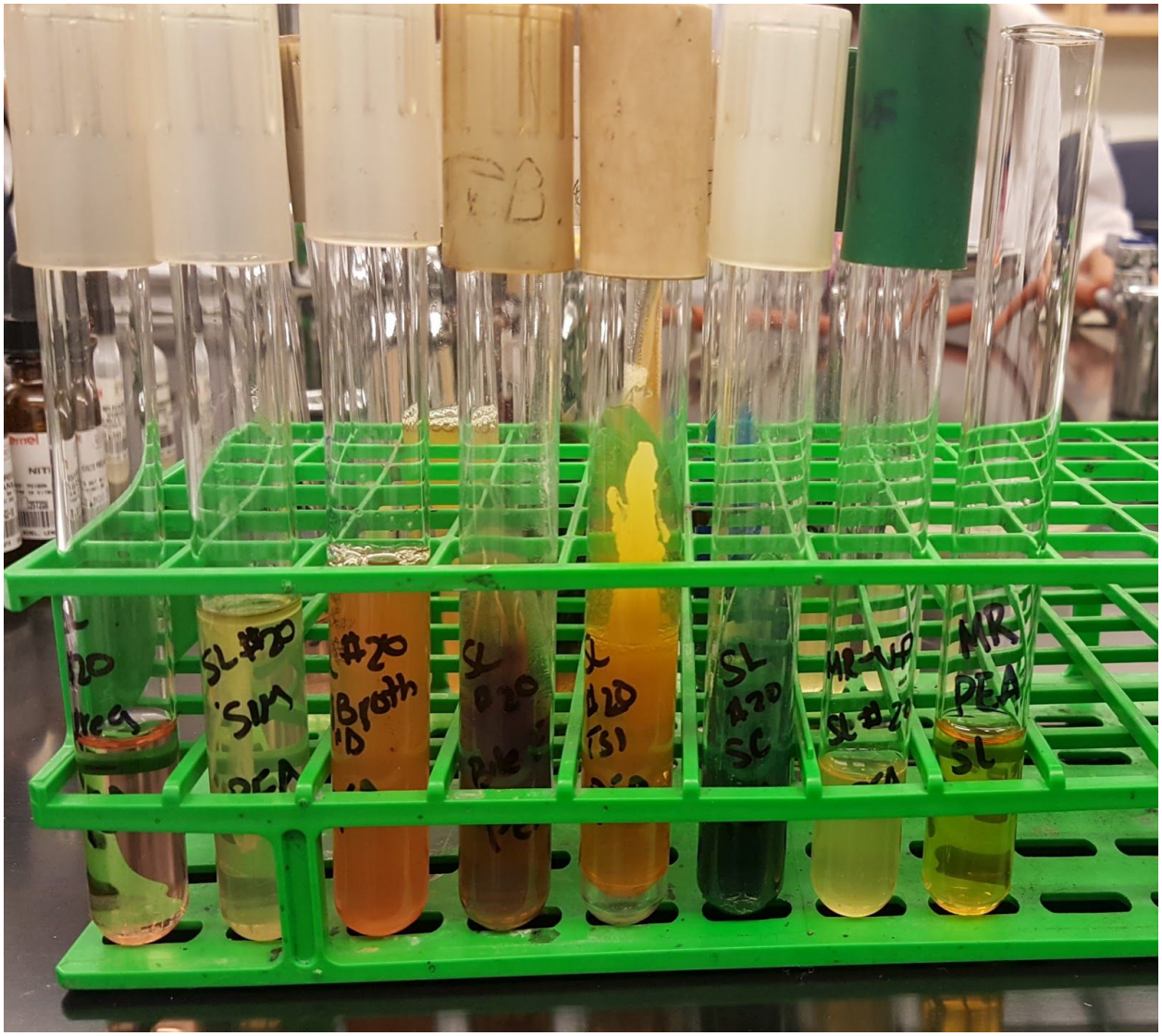

Figure 6 shows the above stated results for the PEA culture.

Discussion/Conclusion

Based on my results, I believe that the unknown gram positive bacteria is *Staphylococcus aureus*. My choice is based mainly on the following: the gram staining revealed purple cocci, the culture of the mannitol salt agar plate turned yellow which is indicative of an organism that can tolerate a high amount of salt, the TSI test revealed an organism which was a fermenter of glucose and/or lactose and sucrose along with

gas formation, the organism was also positive for catalase, indole was negative, nitrate reductase was positive.

Staphylococcus aureus is part of the normal biota of the body. It can however, also be very dangerous pathogen when not found in the correct place. Some strains have also become resistant to antibiotics. *S.aureus* can cause a wide range of mild illness and skin infections, but it can also cause life threatening ones such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. PEA should be used routinely for isolation in gram positive aerobes [4].

I believe the unknown gram negative bacteria is *Enterobacter aerogens*. The main reasons I chose this bacteria are: the gram staining revealed small pink bacillus, the bacteria was a lactose fermenter as shown on the MacConkey agar plate. When gram-negative bacilli ferment lactose, mixed-acid byproducts are formed. These acids cause a localized decrease in pH, producing pink-red colonies [5]. It was positive for citrate, positive for the fermentation of glucose and/or lactose and sucrose with gas production, and it was negative for indole, motility was also noted.

Enterobacter aerogens is part of the normal biota of the GI tract. It can grow both aerobically and anaerobically and can also be found in soil, water and dairy. It is an opportunistic pathogen which can cause gastrointestinal infections, urinary tract infections, skin and soft tissue infections, respiratory infections, and adult meningitis. It is also source of many nosocomial infections.

References

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