Bacterial Unknowns Project

Your Gram negative bacteria

Observations and Interpretations

Isolation Procedure *Record all activities associated with the isolation of your Gram-ve organism to pure culture. Always include the date, source of the inoculum, type of media used, incubation temperature, and any other relevant information.*

Date	Activities Associated with the Isolation of the Gram-ve Organism to Pure Culture
Sept 14, 2021	*Subculturing a Primary Inoculum* – Using a cotton swab, a primary inoculum was made of the mixed broth culture, containing both Gram-ve and Gram+ve bacteria, on a Blood Agar plate.
	Streaking Plates for Isolated Colonies – With a sterilized and cooled loop, the Blood Agar plate was 4-way streaked to dilute and spread out the inoculum. Plates were then incubated in an inverted position, at 35°C for 24 hours.
Sept 21, 2021	*Preparation of Smears from Solid Media* – Using a sterilized and cooled loop, sterile saline was aseptically placed on a microscope slide. Using a sterilized and cooled pick, a small amount of the surface growth of a single colony of the bigger sized colonies, from the previous week's incubated Blood Agar plates, was removed. The organism was then emulsified, using a pick, into the drop of saline on the slide. Next, the slide was air-dried at room temperature. When the slide was dry, it was fixed by quickly passing it with the smear facing upwards through the cooler portion of the flame slowly 3 or 4 times (about 75°C).
	Preparation of the Gram Stain – The smear from the previous activity was flood fixed with crystal violet (primary stain), rinsed with water, flooded with Gram's iodine, rinsed with water, decolorized with acetone until no excess blue color remained, rinsed with water, flooded with basic fuchsin, left for 30 seconds, rinsed again with water, and lastly blotted dry with *Bibulous paper*.
	Analysis of the Gram Stain – The smear was put under a microscope and the gram reaction, relative size, shape, and cell orientation were noted. These microscopic morphologies allowed for the differentiation between the Gram-ve and Gram+ve bacteria.
	Streaking Unknowns for Isolated Colonies – After examining the plate for the appearance of two types of colonies, a sterilized and cooled pick was used to remove the top of one of the colonies of the Gram-ve bacteria. A streak plate was prepared with this bacterium by applying a 4-way streaking technique on a new Blood Agar plate. The plate was then incubated at 35°C for 24 hours. Pure culture was obtained and the Gram-ve bacteria was successfully isolated.

Preliminary Observations

Macroscopic morphology - colony morphology (and medium used)

After incubating the Blood Agar plate, containing the mixed broth culture, inoculated on Sept 14, 2021, I was able to obtain two different looking types of bacterial colonies on my mixed unknown Blood Agar plate. The two different bacterial colonies were my Gram-ve and Gram+ve bacteria. Both bacteria were differentiated by size (small vs big).

In my case, the Gram-ve bacteria was the bigger sized bacteria (see below). The colonies were 0.6mm in diameter, nonpigmented with an off-white/greyish tint, and non-diffusible. The optical property of this colony was opaque with a translucent border around each individual colony. The bacteria had a mucoid texture. There was no odour. The colonies had a circular shape, a smooth, entire margin, and a convex elevation (typical colony).

Microscopic morphology - Gram Stain appearance

The gram stain of the bigger bacterial colonies was pink in color, small, and bacillus (rod shaped). Because the bacterial colonies were pink, this is the Gram-ve bacteria of my unknown bacteria. The cell orientation of the Gram-ve bacteria was pleomorphic as it contained single, diplobacillus, and streptobacillus (chain) orientations.

Differential Tests

Include all pertinent information. Cut or paste test entries as necessary. This log must be kept current.

Test #1: Nutritional Requirements and Classification of Media

Result and Comments: For my Gram-ve bacteria, there was no growth on the 1.5% Agar, L+++ growth on the Mueller Hinton Agar, M++ growth on the Blood Agar, L+++ growth on the Chocolate Agar, and M+ growth on the MacConkey Agar. Because there was no growth on the 1.5% Agar, the bacteria are fastidious. On the Blood Agar, there was a very cloudy but colorless area surrounding the colonies meaning that this bacterium has a partial beta hemolysis. On the MacConkey Agar, the color of the M+ growth is pink meaning that this Gram-ve bacteria is a lactose fermenter.

Test #2: Effect of O2, CO2, and AnO2 Tension on Growth

Result and Comments: For the Gram-ve bacteria, there was L++ growth in the aerobic conditions (O2), L++ growth in +CO2 conditions, and M+++ growth in anaerobic conditions (AnO2).

Test #3: Multiple Sugar Fermentation Testing, The Triple Sugar Iron (TSI) stab/slant – Used to demonstrate the fermentation of D-glucose, lactose, and/or sucrose. Sodium thiosulphate is a source of sulphur and ferrous sulfate is the indicator used to demonstrate H2S production.

Result and Comments: There was a yellow slant and yellow butt (Acid/Acid) in the tube indicating that the bacteria in the slant and butt are glucose, lactose, and/or sucrose fermenters. There were

some cracks and bubbles in the medium indicating that there was gas production. Lastly, there was no blackening in the butt of the tube indicating that there was no H2S production.

Test #4: Citrate Utilization, Citrate Agar Slant – Used to determine whether the organism can utilize citrate as a sole carbon source, inorganic ammonium salts as a sole source of nitrogen and the indicators bromothymol blue.

Result and Comments: The slant color was green and there was no growth of the culture. This means this bacterium is citrate negative.

Test #5: Methyl Red Test and Voges Proskauer (MR-VP) – Used to determine if the organism can produce large amounts of acid by mixed acid fermentation and to determine the production of acetyl methyl carbinol (a product of butanediol fermentation).

Result and Comments: In the Methyl red test portion, the color of the tube was bright red indicating a positive result. In the Voges-Proskauer test portion, there was no change (negative) in color indicating that Acetyl methyl carbinol was absent. Therefore, MRVP is +/-.

Test #6: ONPG Test (Ortho-Nitrophenyl-B-D-Galactoside) – Used to determine the ability of organisms to ferment lactose.

Result and Comments: This bacterium is ONPG positive as the color of the observation was pale to dark yellow. This means that this bacterium is a lactose fermenter.

Test #7: Amino Acid Utilization, The MIO Test – Allows testing of three different reactions in one tube inoculation: motility, indole production, and ornithine decarboxylation.

Result and Comments: The motility of this test was positive as there was cloudy growth throughout the medium. There was no indole production (negative) as the medium was pale yellow/no change. The Ornithine decarboxylation was positive as the medium had a purplish, greyish color. Therefore, the MIO results are as follows: +/-/+.

Test #8: Urea Utilization, Urease Activity – Used to determine whether the bacteria possess the enzyme urease.

Result and Comments: The urease results were negative as there was a peach slant and butt. The reaction was negative. This means that the pH was 6.8 or less.

Test #9: Enterotube Activity – A rapid technique for identification of Enterobacteriaceae and other non-fastidious gram-negative bacilli.

Result and Comments: The results of the enterotube chart provided me with the specified number of my bacteria. Upon looking this number up in the database provided in the lab, I was able to identify my unknown Gram-ve bacteria. The number I got based on the results of my enterotube was #7340. This number corresponds to the bacteria, *Escherichia coli*.

Test #10: Comparing Unknown Bacteria to Known Provided in Lab

Result and Comments: We were also provided with labelled petri dishes of the different possible bacteria that our unknowns could be. When I compared my Gram-ve bacteria, I found that it perfectly resembled the *E. coli* petri dish in the lab. This further confirmed my analysis.

My unknown is *Escherichia coli*

Discussion

Thoroughly describe your rationale for your conclusion. Are all results as expected? Marks awarded will relate to the rigour of your argument. You are encouraged to consult outside sources of information to support your argument. Provide citations if applicable.

This unknown bacterium is *Escherichia coli;* a gram-negative, rod-shaped bacteria. I conclude with this identification due to the following analyses of the different tests listed above. Upon obtaining a pure culture of the bacteria, ten different tests were done. Each test brought my conclusion one step closer, and it is important to analyze each test and see what the results of these tests identify about this Gram-ve bacteria.

For Test #1, the bacteria showed growth on all the different types of agars except 1.5% Nutrient Agar. The pink growth on the MacConkey plate indicated that this bacterium is a lactose fermenter. On the Blood Agar plates, there were very cloudy but colorless areas surrounding the colonies. This demonstrated a partial beta hemolysis. These results were as expected. In Test #2, *E. coli* was able to grow on all three conditions (O2, +CO2, AnO2). This was expected since *E. coli* is a facultative anaerobe which means that the bacteria can grow in both the presence and absence of oxygen (Oleg N. Murashko and Sue Lin-Chao 2017).

Test #3 displayed that the bacteria is a glucose, lactose, and/or sucrose fermenter, it produces gas, and does not produce H2S. This is expected because when *E. coli* ferments sucrose, it lets out carbon dioxide. *E. coli* is also H2S negative as it "cannot easily oxidize H2S to reactive sulfane sulfur and the reduction of reactive sulfane sulfur by cellular thiols is not likely a major route for H2S production" (Li K et al. 2019). Test #4 indicated that *E. coli* is citrate negative. This was also expected since the preferred carbon source for *E. coli* is glucose not citrate, as learned in class.

As expected, Test #5 demonstrated that the organism can produce large amounts of acid by mixed acid fermentation but cannot produce acetyl methyl carbinol. As seen before during test #1, *E. coli* is a lactose fermenter. Because of this, during the ONPG test (test #6), the color of the observation was pale to dark yellow. This further confirmed that this bacterium is a lactose fermenter.

Test #7 provided information on the motility, indole production, and ornithine decarboxylation. These three components help determine whether the organism is part of the *Enterobacteriaceae* family. Prior to starting this project, we were told that our Gram-ve bacteria will be from the *Enterobacteriaceae* family. So, when test #7 also indicated these results, there were no surprises. Test #8 indicated that the bacterium is urease negative. This was expected since *E. coli's* preferred nitrogen source is ammonia, not urea (Anat B. et al 2016).

Lastly, tests #9 and #10 were both tests in which we compared our results with results given in the lab. These were the final identifications for the Gram-ve bacteria. Once my results were compared with the enterotubes and petri dishes provided in the lab, I found that my unknown bacteria, that I was suspecting was *E. coli*, perfectly resembled the *E. coli* petri dish and enterotube in the lab. This further confirmed my analysis. In conclusion, all the results were as expected, and I was able to successfully identify my Gram-ve bacteria.

Your Gram Positive Bacteria

Observations and Interpretations

Isolation Procedure *Record all activities associated with the isolation of your Gram+ve organism to pure culture. Always include the date, source of the inoculum, type of media used, incubation temperature, and any other relevant information…*

Date	Activities Associated with the Isolation of the Gram+ve Organism to Pure Culture
Sept 14, 2021	*Subculturing a Primary Inoculum* – Using a cotton swab, a primary inoculum was made of the mixed broth culture, containing both Gram-ve and Gram+ve bacteria, on a Blood Agar plate.
	Streaking Plates for Isolated Colonies – With a sterilized and cooled loop, the Blood Agar plate was 4-way streaked to dilute and spread out the inoculum. Plates were then incubated in an inverted position, at 35°C for 24 hours.
Sept 21, 2021	*Preparation of Smears from Solid Media* – Using a sterilized and cooled loop, sterile saline was aseptically placed on a microscope slide. Using a sterilized and cooled pick, a small amount of the surface growth of a single colony of the smaller sized colonies, from the previous week's incubated Blood Agar plates, was removed. The organism was then emulsified, using a pick, into the drop of saline on the slide. Next, the slide was air-dried at room temperature. When the slide was dry, it was fixed by quickly passing it with the smear facing upwards through the cooler portion of the flame slowly 3 or 4 times (about 75°C).
	Preparation of the Gram Stain – The smear from the previous activity was flood fixed with crystal violet (primary stain), rinsed with water, flooded with Gram's iodine, rinsed with water, decolorized with acetone until no excess blue color remained, rinsed with water, flooded with basic fuchsin, left for 30 seconds, rinsed again with water, and lastly blotted dry with *Bibulous paper*.
	Analysis of the Gram Stain – The smear was put under a microscope and the gram reaction, relative size, shape, and cell orientation were noted. These microscopic morphologies allowed for the differentiation between the Gram-ve and Gram+ve bacteria.
	Streaking Unknowns for Isolated Colonies – After examining the plate for the appearance of two types of colonies, a sterilized and cooled pick was used to remove the top of one of the colonies of the Gram+ve bacteria. A streak plate was prepared with this bacterium by applying a 4-way streaking technique on a new Blood Agar plate. The plate was then incubated at 35°C for 24 hours. Pure culture was obtained and the Gram+ve bacteria was successfully isolated.

Preliminary Observations

Macroscopic morphology - colony morphology (and medium used)

After incubating the Blood Agar plate, containing the mixed broth culture, inoculated on Sept 14, 2021, I was able to obtain two different looking types of bacterial colonies on my mixed unknown Blood Agar plate. The two different bacterial colonies were my Gram-ve and Gram+ve bacteria. Both bacteria were differentiated by size (small vs big).

In my case, the Gram+ve bacteria was the smaller sized bacteria (see below). The colonies were 0.3mm in diameter, nonpigmented with an off-white/greyish tint, and non-diffusible. The optical property of this colony was opaque. The bacteria had a mucoid texture. There was no odour. Some colonies had a circular shape while others had an irregular shape. The colonies had a filamentous margin. The circular shaped colonies had a convex elevation while the irregular shaped colonies had a flat elevation.

Microscopic morphology - Gram Stain appearance

The gram stain of the smaller bacterial colonies was purple in color, small, and coccus (circular) shaped. Because the bacterial colonies were purple, this is the Gram+ve bacteria of my unknown bacteria. The cell orientation of the Gram+ve bacteria was staphylococcus.

Differential Tests

Include all pertinent information. Cut or paste test entries as necessary.

Test #1: Nutritional Requirements and Classification of Media

Result and Comments: For my Gram+ve bacteria, there was no growth on the 1.5% Agar, M++ growth on the Mueller Hinton Agar, S++ growth on the Blood Agar, M+++ growth on the Chocolate Agar, and S+ growth on the MacConkey Agar. Because there was no growth on the 1.5% Agar, the bacteria are fastidious. On the Blood Agar, there was no haemolysis meaning this bacterium has a gamma hemolysis. On the MacConkey Agar, the color of the S+ growth is yellow meaning that this Gram+ve bacteria is a lactose non-fermenter.

Test #2: Effect of O2, CO2, and AnO2 Tension on Growth

Result and Comments: For the Gram+ve bacteria, there was M++ growth in the aerobic conditions (O2), S++ growth in +CO2 conditions, and S++ growth in anaerobic conditions (AnO2).

Test #3: DNAse test – Used to determine whether the bacteria possess the extracellular enzyme DNAse (endonuclease) which can break down DNS to polynucleotides and nucleotides. Uses two indicators: toluidine blue and methyl green.

Result and Comments: There was a yellow zone around the growth with methyl green. This indicates that the bacteria is DNAse positive.

Test #4: Catalase Test – Used to detect the production of catalase enzyme in the organism.

Result and Comments: There was a positive reaction as there were obvious gas bubbles produced on the glass slide. As per the Gram+ve chart provided in Lab 6 Day 2 manual, because this bacterium reacted positively to this test, this bacterium belongs to the *Staphylococcaceae* family.

Test #5: Slide Coagulase Test - Used to identify the presence of bound coagulase in the organism.

Result and Comments: There was no clumping on either side of the slide. Therefore, this was a negative result. As per the Gram+ve chart provided in Lab 6 Day 2 manual, because it is a negative result, this is the *Staphylococcus epidermis* bacteria.

Test #6: Comparing Unknown Bacteria to Known Provided in Lab

Result and Comments: We were also provided with labelled petri dishes of the different possible bacteria that our unknowns could be. When I compared my Gram+ve bacteria, I found that it perfectly resembled the *Staph epidermis* petri dish in the lab. This further confirmed my analysis.

My unknown is *Staphylococcus epidermis*

Discussion

Thoroughly describe your rationale for your conclusion. Are all results as expected? Marks awarded will relate to the rigour of your argument. You are encouraged to consult outside sources of information to support your argument. Provide citations if applicable.

This unknown bacterium is *Staphylococcus epidermis;* a gram-positive, circular-shaped bacteria. I conclude with this identification due to the following analyses of the different tests listed above. Upon obtaining a pure culture of the Gram+ve bacteria, six different tests were done. Each test brought my conclusion one step closer, and it is important to analyze each test and see what the results of these tests identify about this Gram+ve bacteria.

For Test #1, the bacteria showed growth on all the different types of agars except 1.5% Nutrient Agar. The yellow growth on the MacConkey plate indicated that this bacterium is not a lactose fermenter. On the Blood Agar plates, there were no signs of hemolysis meaning that this bacterium had a gamma hemolysis. As per literature, *Staphylococcus epidermis* is a lactose fermenter, but it still showed yellow growth on the MacConkey Agar. In Test #2 *Staphylococcus epidermis* was able to grow on all three conditions (O2, +CO2, AnO2). This was expected since *Staphylococcus epidermis* is a facultative anaerobe which means that the bacteria can grow in both the presence and absence of oxygen (Uribe-Alvarez C. et al 2015).

Test #3 indicated that the bacteria is DNAse positive meaning that the bacteria possess the extracellular enzyme DNAse (endonuclease) which can break down DNS to polynucleotides and nucleotides. This was also as expected. Test #4 demonstrated that the bacteria produce catalase enzyme. Prior to starting this test, we were given a Gram+ve stain chart describing the characteristics of the bacteria in correspondence to the outcomes of each test. In the chart, it was written that if our Gram+ve bacteria had a positive catalase test, the bacteria will be from the *Staphylococcaceae* family.

Test #5 identified the absence of bound coagulase in the organism. There was no clumping on either side of the slide meaning this was a negative result. As per the Gram+ve chart provided in Lab 6 Day 2 manual, because it is a negative result, this is the *Staphylococcus epidermis* bacteria.

Lastly test #6, was a test in which we compared our results with results given in the lab. These were the final identifications for the Gram+ve bacteria. Once my results were compared with the petri dishes provided in the lab, I found that my unknown bacteria, that I was suspecting was *Staphylococcus epidermis*, perfectly resembled the *Staphylococcus epidermis* petri dish in the lab. This further confirmed my analysis. In conclusion, all the results were as expected, and I was able to successfully identify my Gram+ve bacteria.

References

Anat B., Junyoung O., Benjamin D., Erez D., Joshua D., and Uri A. 2016. Glucose becomes one of the worst carbon sources for E.coli on poor nitrogen sources due to suboptimal levels of cAMP. Scientific Reports. Available from https://www.nature.com/articles/srep24834 [accessed Nov 12, 2021].

Li K, Xin Y, Xuan G, Zhao R, Liu H, Xia Y and Xun L, 2019. Escherichia coli Uses Separate Enzymes to Produce H2S and Reactive Sulfane Sulfur From L-cysteine. Front. Microbiol. 10:298. doi: 10.3389/fmicb.2019.00298.

Oleg N. Murashko and Sue Lin-Chao, 2017. Escherichia coli responds to environmental changes using enolasic degradosomes and stabilized DicF sRNA to alter cellular morphology [online]. PNAS. Available from: https://www.pnas.org/content/pnas/114/38/E8025.full.pdf [accessed Nov 12, 2021].

Uribe-Alvarez C., Chiquete-Félix N., Contreras-Zentella M., Guerrero-Castillo S., Peña A., and Uribe-Carvajal S. 2015. Staphylococcus epidermidis: metabolic adaptation and biofilm formation in response to different oxygen concentrations, Pathogens and Disease. Oxford Academic. https://doi.org/10.1093/femspd/ftv111.