Unknown Identification II

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Abstract

This lab was to allow the students to identify one of many unknown microorganisms that were randomly selected by each student through the usage of many different biochemical tests. These methods involve first forming a dichotomous key that will outline each test and the results of each test in order to identify the unknown. Identification of the microorganism started with ten known microorganisms and was placed accordingly to their own unique characteristics based off of a chart provided to the students. The results indicated that there could only be one correct microorganism and correct interpretation was needed to identify the unknown. The unknown microorganism was correctly identified as *Salmonella typhimurium* through the usage of only three biochemical tests and through three control tests. Without using a dichotomous key all test would have been needed to have been preformed and interopereated which would have taken over a month.

Summary of antimicrobial studies?
Introduction

The purpose of this lab is to correctly identify an unknown microorganism out of a list of known microorganisms. All microorganisms the students were told are all classified as gram negative, rod shaped microorganisms. This is easily confirmed by a simple stain test this will make the gram negative a pinkish color rather than a purple color. First the students must complete their own dichotomous key and have it checked by either Carolyn (our lab assistant), by Dr. Singleton, or by Dr. Mathers who was filling in for Dr. Singleton for a short time. This must be done first in order to continue with the project. This was divided up by different test preformed and the responses. These tests were formed in question form and the responses were simple yes or no answers as much shown in the results section of this report. This method was very useful in utilizing the next test that must be performed and help identifying our unknown microorganism.

After the formation of the dichotomous key which must not start with the lactose test, therefore forcing every student within the lab to take a unique route in order to identify their unknown microorganism. Then the first biochemical test was preformed starting with the hydrogen sulfide test or known as the SIM test. This test also was accompanied but the gram stain test to confirm that each unknown microorganism was indeed a gram negative cell. Also a MacConky’s agar was performed in order to determine whether or not the unknown ferments lactose or not. If the colonies turn a slight pink it is an indication that the unknown ferments lactose however, if the colonies still remains a whitish color the microorganism does not ferment lactose.
With all these test preformed the unknown microorganism was correctly identified as *Salmonella typhimurium*. The history of salmonella starts with a man by the name of Daniel Elmer Salmon who was the administrator for the USDA research program even though a man by the name of Theobald Smith was the man who actually discovered it actual bacterium. This became about when both looking for a cause of hog cholera and would learned that this microorganism did not cause any symptoms in pigs. But later found that this microorganism causes an infection within humans (2). This small microorganism ranges in size from 2-5 micrometer in length and roughly around 0.7-1.5 nanometer in diameter. According to the website rapidmicrobiology.com the type of salmonella that is most common is *Salmonella typhimurium* which mainly induces vomiting, and diarrhea which can cause massive dehydration (3). This has also been more prevalent in both the young such as infants to early childhood to the elderly (3). In short those with a low or weak immune system, Salmonella often gain their energy from a process called oxidation and reduction using organic sources. One of the productions of salmonella is hydrogen sulfide (2). This is also found in Bauman’s textbook saying that the microorganism produces sulfides when placed into a sulfide rich media. This would be proven positive if and only if the agar turns a black color with some precipitation on the top.

MacConky’s agar is a great way to test whether or not salmonella produces lactose. This according to textbookofbacteriology.com salmonella does not. Mainly because of the fact that salmonella does not have any of the means of making lactose nor would it need any of the materials needed to make lactose and therefore cannot and will ferment lactose (4). However, it has been found to make glucose if under the right conditions. This is because the DNA of salmonella is closely related to the *Escherichia* genus which can in fact make glucose (2). All tests that were performed on salmonella agree with all of the previous experiments done by other...
professionals and support not only the findings found over the past couple of weeks but also and in support of the findings of those before performing these experiments.

**Methods**

As mentioned in the introduction a dichotomous key was first formed in order to help separate each microorganism by the means of various biochemical tests. The first biological test that was performed was the hydrogen sulfide also known as a SIM test which involves a certain type of media needed called also a SIM media with a reagent within the media being Iron (\( \text{Fe}^2+ \)). With the needle in hand and the microorganisms on the needle just stab straight in straight out of the gel like media, and place it in the incubator at about 35°C for approximately 24 hours. If the microorganism does turn the clear yellow agar into a black agar the unknown microorganism produces hydrogen sulfides making the result positive. If the agar remains a clear yellow then the microorganism does not making the result negative. While starting our first biochemical test we also started a Triple Sugar/Kligier’s Iron Agar test or TSIA test for short. A small amount of our unknown microorganism was placed on the needle and stabbed through the agar and placed at the top of the slant. After the test was performed the TSIA test was put aside until the following lab period. Results may vary however what is known is this. If a yellow slant and a yellow butt are present it means that glucose and lactose are fermenting with acid on the slant and in or on the butt. If a red slant appears and a yellow butt is present glucose fermentation with acid production on the slant. If a red slant and butt are prevalent there is no fermentation but peptone catabolized aerobically with alkaline products. If a red slant appears and there is no change to the butt it is the same as before. If there is no change in on the slant or in the butt the organism is growing slowly or not at all. Black precipitate in the agar appears it means that there
is sulfur reduction going on. Last is if there are cracks in or lifting the agar it means that there is gas production.

The next test that was performed in the list was the Urea test. The media for this test is a simple urea broth with a reagent of phenol red. A loop of the unknown microorganism is dipped into the urea broth and placed in the incubator again for at least 24 hours at 35°C. This test helps indicate if the unknown produces any type of urea at all by turning the yellow solution to a hot pink solution. A negative result would still be a yellow or an orange colored solution. The last test that was performed that helped determine the unknown microorganism was the lactose test. The media included phenol red broth and once again the reagent was phenol red. Once a small amount of the unknown microorganism was placed in the solution it was left in the cabinet for 24 hours at room temperature. If this test turned positive a bright yellow color would appear. Along with a gas bubble in the smaller inverted tube that was placed inside the culture tube. This indicates that the microorganism in question produces lactose and releases gas as a byproduct when placed in a red solution that permits the fermentation of lactose. However, if the result is negative the solution would remain red or half yellow half red and no gas bubble would have been produced. The last two sets of tests that were performed were the antiseptic/disinfectants and antimicrobial. The antiseptic/disinfectants used four small pads and labeled A-D. A and B were antiseptics with A being Hibiclens and B had Bactine. C and D were disinfectants with C being Nature Source and D being Clorox. These pads were then placed on an agar with the unknown microorganism spread all over onto the agar. During the next lab period measurements in millimeters were taken from the edge of each pad or disk placed into the agar to where the clear zone ended. These numbers where then recorded into the notebook. Last was the antimicrobial test which followed that same procedure and done on the same day as the
antiseptic/disinfectants. The only difference is with five not four tiny pads were used with known antibiotics on each pad. These pads were already labeled on both front side and back side so no other labeling was needed other than to record it into our notebook. The pads used were E15 (Erythromycin), S10 (Streptomycin), P10 (Penicillin G.), C30 (Chloramphenicol), and K30 (Kanamycin). After recording the antiseptic/disinfectants measurements, measurements from one end of the clear zone to the other end were taken. Once again in millimeters and then rated on the effectiveness of the antibiotic to the unknown microorganism using a chart that was provided to us. Results for these tests are in the following section.

**Results**

A Simple gram stain was performed on the unknown microorganism and was observed to be a light pink in color, small rod shaped microorganisms throughout the whole slide. However nothing can be determined at that time more tests were needed in order to help identify the unknown even further. The next test that was preformed was the Triple Sugar Iron Agar test. This test was a slanted clear yellow agar that from top to bottom appeared red at the top, black mid-section, yellow bottom, and no gas bubbles or cracks were observed inside of the agar. The MacConkey’s agar was then observed and found to have whitish clear colonies over top of a still a red agar.

Antiseptic/Disinfectants test that was performed was to only see which of the four would be best to combat the unknown microorganism. Using the distance from the edge of the pad to the end of the clear zone or the radius of the circle, all measurements are as shown on table 1. Antibiotic followed that same type of procedure the only difference was the measurement style.
The diameter of the zone was measured rather than the radius. All measurements are shown on table 2.

Next was the biological test for these were the main tests done in order to identify the unknown microorganism. First was the hydrogen sulfide test again also known as the SIM test which turned the clear yellow agar into a black agar with some precipitate floating at the top. After 24 hours of incubation at approximately 35°C this is shown in figure 2. The second test that was performed was the urea test. This again after 24 hours of incubation at 35°C the color turned from a yellow to a light orange color. The Third and final test that was performed was the lactose test. This test went from a yellow color to a mixture of a yellow bottom and a red top but with no gas present as shown in figure 4.

**Discussion**

The unknown microorganism was identified through a series of biochemical tests starting with the SIM test and, depending on the result of that test determined which test needed to be performed next. Starting with the SIM test was a good idea because it helped eliminate these six microorganisms: *Serratia marcescens*, *Escherichia coli*, *Porvidencia stuartii*, *Hafnia alvei*, *Klebsiella pneumonia*, and *Enterobacter aerogenes*. The reason for the easy elimination was that the SIM test proved positive for producing sulfides and none of the listed microorganisms can produce any sulfide. This left me with only four microorganisms left. The second test helped determine if the microorganism produced urea at all. The results were negative which helped eliminate *Proteus mirabilis* and *Proteus vulgaris* for those two microorganisms produce urea and therefore were eliminated from the options. With only two microorganisms left and the only test to perform the lactose test helped to see which microorganism it was. With a negative reading on
the lactose test I could safely assume that the unknown microorganism was Salmonella typhimurium. The reason behind that citobacter freundii produces lactose and therefore the test would have been positive but instead was negative and therefore leaving me with salmonella.

The antiseptic/disinfectants test quite honestly didn’t help much the results were still unclear as to what the unknown organism was. The only type of antiseptic that worked was the bactine with a radius of 6mm which was the same for the disinfectant nature source which to my surprise was much stronger than Clorox. One the other hand for antibiotics it was resistant to K30, C30, P10, and E15, yet susceptible to S10 (Streptomycin). This means that the normal penicillin would not even help against which is known for salmonella. All tests performed clearly supports all that is known about salmonella for instance salmonella is known for producing sulfides when place in an appropriate environment. The first test was SIM tests for sulfide producing microorganisms buy turning the yellow agar black with some precipitate at the top. The test was positive and therefore agrees with the fact that salmonella is a producer of hydrogen sulfide when placed in an appropriate media. Salmonella from research is a gram negative rod shape microorganism and from just the appearance that was seen it was gram negative and rod shaped. Therefore agreeing with the fact that salmonella is a gram negative, rod shaped microorganism. According to multiple sources salmonella does not produce any lactose at all which agrees with the lactose test that was performed, however, some sources does say that salmonella can in fact produce glucose which agrees with the TSIA test results. Which had a red slant with some what appeared to be an acidic solution or mucus on the top of the red slant. Therefore the unknown that was tested has been proven to be Salmonella typhimurium through the series of tests that were performed and the fact that the results agree with the reach done by many other professionals beforehand on the microorganism.
Work Cited


Figure 1

Dichotomous Key

1. Does it produce sulfielded?
   - Yes
   - No
2. Does methyl red react?
   - Yes
   - No
3. Does it produce lactose?
   - Yes
   - No
4. Does it produce indole?
   - Yes
   - No
5. Does it have vogues?
   - Yes
   - No
6. Does it produce lactose?
   - Yes
   - No
7. Does it produce citrate?
   - Yes
   - No
8. Does it produce gas?
   - Yes
   - No
9. Is it mobile?
   - Yes
   - No
10. Does it produce lactose?
    - Yes
        - Enterobacter aerogenes
    - No
        - Salmonella typhimurium
        - Citrobacter freundii
        - Proteus mirabilis
        - Proteus vulgaris
        - Serratia marcescens
        - Escherichia coli
        - Providencia stuartii
        - Klebsiella pneumoniae
        - Hafnia alvei
        - Serratia marcescens
SIM Test

Figure 2:
Urea Test

Figure 3
Lactose Test

Figure 4
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<th>Active ingredient</th>
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