

Identifying *Enterobacter aerogenes* from a Mixed Culture of Unknown Gram Positive and Gram Negative Bacteria

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PURPOSE

The focus of this study is to be able to identify an unknown bacterium, specifically a gram negative one, from a mixed culture. This study was done in order to apply the various lab techniques used to identify bacterium that were covered in a microbiology course. The study shows that by combining a number of identification tests in series, a single bacterium can be identified from an unknown mixed culture. It can be concluded that a similar process can be used in other studies to be able to identify various bacterium species.

These tests have been used in previous studies to help researchers and scientists identify the bacterium that is the cause of a disease outbreak. One such example was back in 2001, when an anthrax outbreak occurred and researchers worked to identify the cause of the outbreak (University of Arkansas, Fayetteville, 2003). In addition, other studies have used similar tests in identifying bacteria from positive blood samples (Durso, 2008). There are many, many more examples of various previous studies where similar identification processes are utilized (Dyer, 2005).

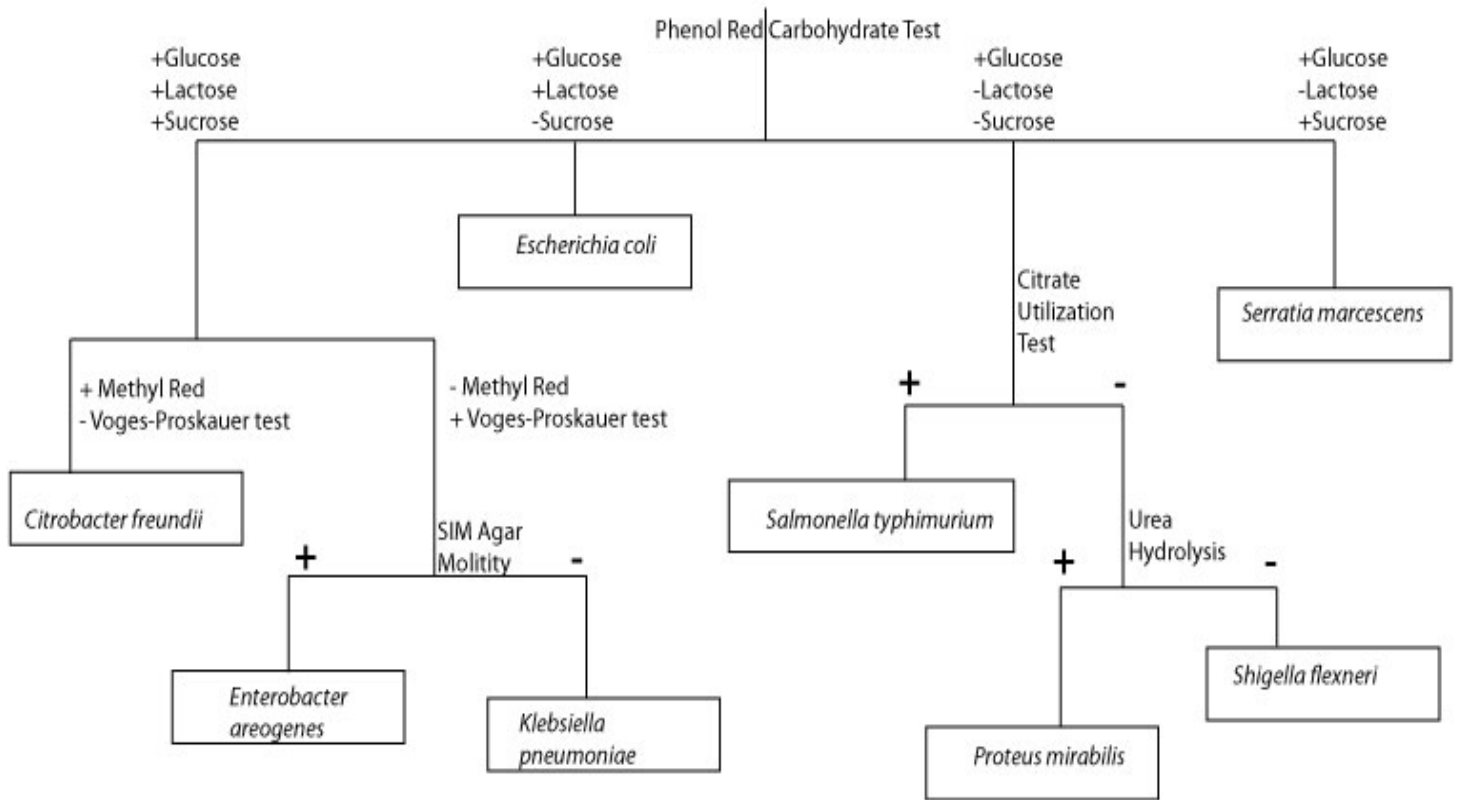
There are many different tests that can help identify bacteria. The biochemical tests used in this study are the Phenol Red carbohydrate, Methyl Red, Voges-Proskaur, Citrate Utilization, SIM agar, and the Urea Hydrolysis tests. The phenol red carbohydrate test is used to see if a bacterium is able to ferment different sugars such as glucose or lactose as well as determine if gas is produced in the process. This allows us to determine if a certain bacterium is able to utilize various carbohydrates. The methyl red test is coupled with the Voges-Proskaur test and helps determine if there is mixed acid fermentation present by detecting the pH level. This test helps

determine if there is acid formation due to fermentation. The Voges-Proskaur test will detect the presence of acetoin, which indicates that there is fermentation of glucose into acetoin and 2,3-butanediol. This test allows the identification of the formation of 2,3-butanediol from glucose. The citrate utilization test checks if a bacterium is able to use citrate as its only source of carbon. The last test used in this study is the SIM agar test. This test is able to identify three different bacterial activities. The SIM agar test is able to check for sulfur reduction, the production of indole from tryptophan substrates, and the motility of a bacterium. This test allows the confirmation of the reduction of sulfur, the production of indole, and the ability for the bacterium to move. These are all of the tests used within this study, but there are more available to use to determine a bacterium.

The unknown bacterium in this study is from the *Enterobacteriaceae* bacteria family. *Enterobacteriaceae* are rod shaped, Gram-negative bacterium (Slonczewski, 2010). Many of these species are motile and flagellated. In addition, they are able to grow singly, in chains, or even in biofilms, many strains, with or without oxygen. It is important to be able to identify bacterium from the *Enterobacteriaceae* group because different strains of a certain bacterium may be beneficial to humans, but other strains can be harmful and cause disease or illness.

In order to properly identify the bacterium from the *Enterobacteriaceae* group, a flow chart was designed (FIGURE 1). Included in this flow chart are the various tests available to be used in this study. The flow chart was designed to identify the bacterium in as few tests as possible. However, other tests are available for confirmation of the bacterium. Based on previous microbiological knowledge, if the various biochemical tests are performed on an unknown bacterium isolated from a mixed culture, then it can be identified as a single bacterium. The identified bacterium should be one of the various Gram negative *Enterobacteriaceae* found in figure 1 and can be confirmed by performing additional tests.

FIGURE 1 – Flowchart to determine unknown *Enterobacteriaceae* bacterium



The study began with an unknown mixed culture, labeled #5, made up of one Gram positive and one Gram-negative bacterium. This culture was inoculated using the quadrant streak method on a nutrient agar plate, in order to obtain isolated colonies of each bacterium. While the culture tube was placed into refrigeration, the agar plate was then incubated at 37 degrees Celsius for 48 hours. After incubation, the plate was then analyzed for two different isolated colonies. With successful isolation of two different bacterium colonies, a gram stain was performed on each of the different isolated colonies and one colony was identified as Gram-negative and the other was identified as Gram-positive. The study was continued with the Gram-negative bacterium by inoculating a second nutrient agar plate with the bacterium from the first plate in order to obtain only Gram-negative isolated colonies. The second agar plate was then incubated at 37 degrees Celsius for 48 hours.

After this second inoculation, the biochemical portion of the testing process began. First, the Phenol Red glucose, lactose, and sucrose tests were done in conjunction with the Methyl Red and Voges-Proskauer test by inoculating the Gram-negative colonies from the second agar plate into the respective test tubes. These test tubes were then incubated for 48 hours at 37 degrees Celsius before reading the tests while the second agar plate was refrigerated. After reading the results of the Phenol Red Carbohydrate tests, the Methyl Red test and the Voges-Proskauer test, the Citrate Utilization and Urea Hydrolysis tests were performed by inoculating colonies from the refrigerated second plate into the respective test tubes and then incubated at 37 degrees Celsius for 48 hours. After the results from this test were read and recorded, the final test, the SIM agar test was performed by inoculating the Gram-negative colony into a test tube and incubating for 48 hours at 37 degrees Celsius. After this final test was performed, enough information was obtained to confirm the identity of the unknown Gram-negative bacterium.

RESULTS

Through the gram stain, various pieces of important microscopic data were obtained (TABLE 1). While organism 1 showed pink and red stained cells, organism 2 showed purple stained cells. The shapes of the two organisms also differed, with organism 1 having rod shaped cells and organism 2 having round cells. The spatial arrangement of both organisms was the same. Both organisms grew singly and spread evenly throughout.

TABLE 1: Summary of Microscopic data on organism 1 and 2

Organism	Color	Shape	Arrangement
Organism 1 (<i>Enterobacter aerogenes</i>)	Pink and Red	Rod shaped	Singly evenly spread
Organism 2	Purple	Round	Singly evenly spread

Many addition results were obtained from using organism 1 in the biochemical tests (TABLE 2). The first round of testing included test tubes that had Phenol Red tests for glucose, lactose, and sucrose as well as the Methyl Red and Voges-Proskaur test. The Phenol Red test for glucose, lactose, and sucrose all turned yellow after incubation as well as had gas within the Durham tube. Once incubation was completed and Methyl Red was added to the respective test tube, the solution within the test tube did not change color. However, after incubation and the two reagents were added to the Voges-Proskaur test tube, the test tube turned red after approximately 30 minutes.

The next round of testing included the Citrate Utilization and the Urea Hydrolysis tests. After the incubation period, the Citrate Utilization test turned a blue color. The Urea Hydrolysis test turned slightly yellow after incubation.

The final round of testing was the SIM Agar test. The test tube was noted to have no color change after incubation. However, there was noticeable outward growth radiating from the original inoculation spot. After adding Kovac's reagent to the test tube, there was no red color present.

Based on the results of the biochemical tests, it can be safely concluded that the unknown bacterium was *Enterobacter aerogenes*.

TABLE 2: Biochemical test results on organism 1 (*Enterobacter aerogenes*)

Organism	Organism 1 (<i>Enterobacter aerogenes</i>)		Organism	Organism 1 (<i>Enterobacter aerogenes</i>)
Phenol Red Glucose	Yellow/ Gas		Citrate Utilization	Blue
Phenol Red Lactose	Yellow / Gas		Indole Production	No Change
Phenol Red Sucrose	Yellow/ Gas		Sulfur Reduction	No Change
Methyl Red	Yellow		Urea Hydrolysis	Slightly Yellow
Voges-Proskaur	Red		Motility	Outward Growth

DISCUSSION:

The gram stain and biochemical tests revealed a lot of information about the bacterium. The bacterium for organism 1 was seen as pink, rod shaped, and growing singly (TABLE 1). These are all characteristics of the *Enterobacteriaceae* group and confirmed that organism 1 was the bacterium of interest in this study.

The Phenol Red Carbohydrate test for glucose, lactose, and sucrose all turned yellow and produced a gas. This indicated a positive result that the unknown bacterium was able to ferment glucose, lactose, and sucrose as a carbohydrate source and producing an acid and gas in that process. The Methyl Red test was negative with no color change. This meant that the unknown bacterium was not capable of the mixed acid fermentation required to lower the pH in the test tube. The Voges-Proskaur test came out positive with a change in color after a period of time, indicating fermentation of glucose to acetoin and 2,3-butanediol. The positive blue result of the Citrate Utilization indicates the ability of the bacterium to use citrate as the sole source of carbon. The negative result of the yellow Urea Hydrolysis indicated there is no urease enzyme present. While the lack of color change in the SIM Agar test with and without the Kovac's reagent indicated there was no sulfur reduction or production of indole, the outward growth in the medium shows that the bacterium is motile.

The biochemical tests confirm that the unknown bacterium that was the focus of this study was indeed *Enterobacter aerogenes*. All of the test results match the profile for *E. aerogenes* exactly with no variation in the expected results. *E. aerogenes* is commonly found within the human gastrointestinal tract. Often, it is not harmful until it moves outside of the tract. *E. aerogenes* is often incurable with drugs. This study can be useful in the future by providing a second study that can be compared to bacteria identification tests.

By using various identification tests in order, *E. aerogenes* was concluded as the Gram-negative bacterium from the unknown mixed bacteria culture.

WORKS CITED

Dyer, Betsey Dexter. "A Brief Guide To Identifying Bacteria In The Field, For Protistologists." *The Journal of Eukaryotic Microbiology* 52.2 (2005): 7S-27S. Print.

Jordan, J. A., J. Jones-Laughner, and M. B. Durso. "Utility Of Pyrosequencing In Identifying Bacteria Directly From Positive Blood Culture Bottles." *Journal of Clinical Microbiology* 47.2 (2009): 368-372. Print.

Slonczewski, Joan, and John Watkins Foster. "18." *Microbiology: an evolving science*. 2 ed. New York: W.W. Norton & Co., 2010. 704. Print.

University Of Arkansas, Fayetteville (2003, May 13). Researchers Use New Method To Accurately Identify Bacteria. ScienceDaily. Retrieved November 13, 2013, from <http://www.sciencedaily.com/releases/2003/05/030513081224.htm>