

Microbiology unknowns lab report assignment



**ASSIGN
BUSTER**

Fifth bacteria is a fermented they will use the sugars to make TAP. If the bacteria is a fermented of lactose/sucrose the EMBED plate we seed will “ clearly differentiate between the colonies of lactose fermenting and non-fermenting microbes. In the same medium sucrose was also included to differentiate between chloroforms that were able to ferment sucrose more rapidly than those that were unable to ferment sucrose” (Chapter & Ala, 2007).

If it is catalane positive it will produce bubbles with the drops of hydrogen peroxide on the bacteria. This will show if the bacteria is an aerobic or rare tolerant anaerobes or true anaerobes. Catalane is an enzyme that will take hydrogen peroxide and break it down to water and oxygen. Merriam Webster 201 3) “ Catalane is thought to be a major defense against hydrogen peroxide” (Eaton & Ma, 1992). We will also determine if the bacteria is stable or non-stable acid ferments.

If it is stable it will produce the acid and it will be a positive result. This test will tell us the pathway of fermentation. Another thing we will determine is that if there will be a presence of citrate permeate. Which will increase the pH level. (Health and Life Sciences Department 2013) By doing these tests we will be able to see how the unknown bacteria reacts to certain conditions. If our results f these lab experiments are conclusive then we should be able to determine what our unknown bacteria is.

Materials and Methods Tube with unknown bacteria in a broth Bunsen burner and Striker Inoculating loop Inoculating needle pen Tape for labeling tubes and plates Test tube rack Dropper/pipette Nutrient Agar Plate Hydrogen

peroxide EMBED Plate Tube of MR..-UP (methyl red) Tube of Simmons citrate methyl red Para film Incubator We started our experiment by inoculating a tube of Methyl Red (MR..-UP) with our unknown bacteria using the aseptic technique and our loop by swirling it in the media. We capped the tube and we then incubated the tube at 37 degrees Celsius for five days then refrigerated until the next class.

We then added five drops of methyl red and gently tapping the tube to distribute. Next we inoculated a Simmons Citrate agar slant using the needle in the aseptic technique. We inserted the needle in the unknown bacteria and then we inserted the needle into the agar to the bottom of the tube and pulled it up along the slope of the slant. We then capped the tube and inoculated the tube at 37 degrees Celsius in an incubator for seven days then refrigerated until the next class. Our third test was on a nutrient agar (AN) plate.

Again using the aseptic technique we streaked our unknown bacteria down the center of the plate in a heavy line using the loop. This plate was sealed with Para film, flipped upside down and incubated at 37 degrees Celsius for 16-24 hours then refrigerated. At our next class we added three drops of hydrogen peroxide using the dropper directly to the bacteria line to test for catalane. We ended our experiment using the Eosin Methyl Blue (EMBED) plate. Using the aseptic technique we streaked the plate with the unknown bacteria with the loop in a T-streak method.

The plate was sealed with Para film, stored upside down and incubated at 37 degrees Celsius for 24 hours then refrigerated Results In the MR..-UP test we

did with the unknown bacteria we found that after adding the methyl red to the MR..-UP Broth it turned red. By the color of the broth with the unknown bacteria turning red we determined that it is a positive result. Image 1 MR..-UP Broth Tube In the next test that we performed was the Simmons Citrate agar slant test. In looking at the tube after the incubation period we determined that the result of this test was negative due to the green color of the agar.

Image 2 Simmons Citrate Agar Slant Tube We then performed a nutrient agar pate test (AN). In this test we were checking for catalane. After adding drops of hydrogen peroxide to the bacterial growth on the plate we notice no bubbles. Once the plate sat for about 5 minutes, we then noticed bubbles on the bacteria growth. The bubbles show a result of catalane. We do not have an image of the oxygen gas being present because initially it did not bubble, so we did not think the results would be positive. Our last test that was performed was the Eosin Methyl Blue agar plate. EMBED) Which showed black/deep purple metallic sheen which indicates a positive result in lactose/sucrose fermentation. Image 3 Eosin Methyl Blue Agar Plate (EMBED)

Discussion We were given a tube of unknown bacteria labeled #3, which after doing several experiments we determined that the unknown bacteria was *Escherichia coli*. The results of these experiments were conclusive with what the *Escherichia coli* bacteria would do in these tests. We did an MR..-UP test which if positive for *Escherichia coli* it would turn red after adding the methyl red. Our results were positive the MR..-UP turned red.

During the Simmons Citrate Agar slant test the *Escherichia coli* would test negative showing a green colored agar after the incubation period. Our

Simmons Citrate Agar was green in color. We performed a Nutrient Agar plate test (AN) in this test we will test for catalane. After dropping the hydrogen peroxide on the bacteria we did not get bubbles at first. After waiting a few minutes we then noticed bubbles, which was a catalane positive result. This also was a result that the *Escherichia coli* bacteria will produce. “ The catalane activity within individual *Escherichia coli* fails to protect against exogenous hydrogen peroxide.

Contrary to earlier reports we find that dilute suspensions of wild-type and catalane-deficient *Escherichia coli* are identical in their sensitivity to hydrogen peroxide, perhaps because even wild-type, catalane positive *Escherichia coli* cannot maintain an internal/external concentration gradient of this highly diffusible oxidant. However, concentrated suspensions or colonies of catalane positive *Escherichia coli* do preferentially survive hydrogen peroxide challenge and can even cross-protect adjacent catalane-deficient organisms. ” (Eaton and Ma, 1992)

The last test we performed was on the Eosin Methyl Blue Agar plate (EMBED). Our test concluded that it was a positive result in lactose/sucrose fermentation. It showed a black/purple metallic sheen. “ *Escherichia coli* colonies grow in the metallic sheen with a dark center”. (Chapter and Ala, 2007). *Escherichia coli* is a bacteria that comes from contaminated food, water or person to person contact. It comes from undercooked meat or vegetables that have been washed with contaminated water. Prevention of this is to wash your hands and make sure there is no cross contamination and thoroughly cook your food.

Most varieties of *Escherichia coli* are harmless or cause relatively brief diarrhea. But, a few particularly nasty strains such as *Escherichia coli* 0157:HA, can cause severe abdominal cramps, bloody diarrhea and vomiting. Complications from *Escherichia coli* are that you may develop a life threatening form of kidney failure called Hemolytic Uremia Syndrome (HUS). " (Mayo clinic) Most people recover within a week. There are no current treatments for this infection, just avoiding dehydration is a big factor. In conclusion all of the test that were performed resulted that this unknown bacteria is *Escherichia coli*.