

# Microbiology unknown assignment



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Autumn White Biology 258-05 November 26, 2012 Unknown #19

Interchangeable Interrogated arrogates ABSTRACT The objective of this report was to identify an unknown microorganism through several differential media tests. Over the course of a couple weeks, ten tests were performed. First, a gram stain was performed, indicating the bacterium was gram negative. An aerodonetics test determined that the bacterium was a facultative anaerobe. Next, a negative result in the methyl red test indicated that no mixed acid fermentation occurred. The Dense test was performed and yielded a positive result.

The SIMI test provided two outcomes, that the bacterium did not reduce sulfur nor produce indolent from thyrotrophic. Afterwards, the bacterium was determined to be positive for lysine decertification and citrate. The purple broth and triple sugar iron tests both indicated gas production. The purple broth test was positive for fermentation, and the triple sugar iron test indicated that the bacterium fermented glucose and sucrose. Finally, the bacterium was areas negative. Based on the results of the tests performed, the microorganism was identified as Interchangeable Interrogated arrogates.

Microbiology is a specialized area of biology that places an emphasis on microorganisms. Several microorganisms are included in this subject, such as bacteria, fungi, algae and protozoa. The most important fact that should come out of microbiology is the “ profound influence” that microorganisms have on the aspects of earth (Cowan, 2012). The objective of this project was to identify an unknown bacterium through several differential tests.

Identifying an organism can be very beneficial in the field of medicine.

Knowing what an organism is can indicate its pathology and treatment.

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These tests are designed to find out about an organism's metabolism and other various characteristics. For this project, ten differential tests were performed: gram stain, aerodonetics, methyl red, Dense, SIMI, lysine decertification, Simmons citrate, purple broth, triple sugar iron agar and areas tests. By identifying microorganisms, scientists have been able to identify causative agents for many infectious diseases. In addition, they have discovered many distinct connections between the organisms and diseases whose causes were previously unknown (Cowan, 2012). MATERIALS AND METHODS

Aseptic technique is the sterile technique that decreases the possibility of contamination of the culture, the sterile medium and the surroundings. This technique is used throughout each procedure by the practice of flaming loops between inoculations and having a sterilized working environment.

Gram Stain 1. A drop of water was added to a slide, and the bacterium was smeared using a flamed loop 2. After the slide dried, the primary stain (crystal violet) was added to the see Tort one minute 3. Tater ten see was release Walt Olsten water, ten mordant (iodine) was added for one minute 4. After the slide was rinsed with the decolonize (ethanol), the secondary stain/countersink (seafaring) was added for one minute 5. After the slide was rinsed with distilled water, the slide was blotted dry with a paper towel and observed under oil immersion with a xx lens Possible observations: If the bacterium is Gram positive, it will appear purple. If the bacterium is Gram negative, it will appear red. The Gram positive bacterium traps the dye with its thick poetically layer. Aerodonetics Test 1. The bacterium was inoculated using a flamed loop into fluid technologically medium .

The tube was incubated at ICC for 24 hours Possible observations: If the bacterium is a strict aerobic, it will reside mostly on the top of the medium. If the bacterium is a facultative anaerobe, it will disperse throughout the medium. Methyl Red Test 1. The bacterium was inoculated with a flamed loop into the broth containing glucose, peptone and phosphate buffer 2. The tube was incubated at ICC for 24 hours 3. After incubation, approximately 6-8 drops of methyl red were added Possible observations: If mixed acid fermentation occurs, the broth will turn red.

If mixed acid orientation does not occur, the broth will not change its color.

Dense Test 1. The bacterium was inoculated with a flamed loop onto agar containing peptides for soybeans, casein, NCA and DNA 2. The plate was incubated at ICC for 24 hours Possible observations: If there is a clearing in the agar around the growth, then Dense is present. If there is not a clearing in the agar, Dense is not present. SIMI Test: Tests for sulfur reduction, indole production from tryptophan, and motility 1. The SIMI slant was stabbed with a flamed needle inoculated with the bacterium 2.

The slant was incubated at ICC for 24 hours 3. After incubation, Kovacs' reagent was added. The Kovacs' reagent will react with any indole present Possible observations: If sulfur was reduced, there will be black in the medium. If sulfur was not reduced, there will not be black in the medium. If tryptophan is broken down into indole and pyruvate, there will be red in the alcohol layer of Kovacs' reagent. If tryptophan does not break down, then the reagent color will be unchanged. If there is growth radiating from the stab line, there is motility. If there is no radiating growth, there is no motility.

**Lysine Destroyable:** Tests for the presence of lysine destroyable, which breaks down an amino acid, and glucose fermentation 1. The bacterium was inoculated with a flamed loop into broth containing potent, glucose, pH indicator processes purple, and pyramidal phosphate 2. After inoculation, mineral oil was added to promote Termination 3. I nee Drown was Incubated at 3/c Tort 24 noirs Possible observations: If there was no glucose fermentation or decertification, then there will be no color change. If glucose fermentation occurs, but decertification does not, then the broth will turn yellow.

If glucose fermentation and decertification occur, then the broth will be purple. **Simmons Citrate Test:** Tests for the microorganism's ability to use citrate as a sole carbon source and the presence of citrate permeate 1. The bacterium was inoculated with a flamed needle and streaked onto a slant containing sodium citrate, ammonium dehydrogenate and brotherly blue 2. The slant was incubated at ICC for 48 hours Possible observations: If citrate was utilized by the microorganism, then the slant will be blue or there will be growth from the streak.

If citrate was not utilized, there will e no color change in the slant, and there will be no growth. **Purple Broth Test:** Tests for acid production from sugar fermentation 1. The bacterium was inoculated with a flamed loop into purple broth medium containing lactose, potent and processes purple 2. The broth was incubated at ICC for 48 hours Possible observations: If there was fermentation with Just acid products, the broth will be yellow. If there was fermentation with acid and gas products, the broth will be yellow, and there

will be bubbles in the Durham tube. If there is no fermentation, the broth will be purple.

The broth will also be turbid if there was degradation of protein with alkaline end products. Triple Sugar Iron Test: Differentiates based on glucose fermentation, lactose/sucrose fermentation, sulfur reduction and gas production 1. The bacterium was inoculated with a flamed needle, stabbed into the agar butt and streaked onto the slant 2. The slant was incubated aerobically at 37°C for 24 hours Possible observations: If the microorganism ferments glucose only, the tube will have a yellow butt and a red slant. If the microorganism ferments glucose and either sucrose or lactose, the tube will have a yellow butt and a yellow slant.

If the microorganism does not ferment any of the sugars, the tube will have a red butt and a red slant. If there is sulfur reduction, there will be black in the medium. If there is gas production, there will be cracks in the agar. Urea Test: Differentiates based on the ability to hydrolyze urea with the enzyme urease 1. The bacterium was inoculated with a flamed loop into urea broth containing yeast extract, potassium phosphate, urea and phenol red 2. The broth was incubated at 37°C for 24 hours Possible observations: If there was urea hydrolysis, the broth will be pink. If there was not urea hydrolysis, the broth will be orange or yellow.

RESULTS Test Result -ram stall I see Decelerate appeared rear unaware 011 Immersion Its cell morphology was single bacillus Aerodonetics Test The bacterium was growing throughout the medium, but was more dense near the top Methyl Red Test The broth appeared light orange Dense Test There

was a clearing around the inoculation SIMI Test The medium did not change color with the addition of Kovacs' reagent, nor did it have any black residue Lysine Destroyable Test The broth was purple Citrate Simmons Test The slant was blue and there was growth around the inoculation Purple Broth Test The broth was yellow, and there were bubbles in the Durham tube Triple Sugar Iron Tested agar had a yellow butt and red slant, and there were large cracks in the agar Areas Test The broth was orange Based on the tests performed, the pre-emptive identification of the organism is *Flowchart* *Vacillate* *Antibacterial* *Macroeconomic* *Pseudoscience* *Mycobacterium* *Deontological* *Strict* *Arroba* *Facultative* *Anaerobe* *Escherichia coli* *Aeron's* *isobar* *Providence* *Stuart* *Interrogated* *arrogates* *Cacciatore* *friendly* *Seriate* *marches* *Aeron's* *isobar* *Interrogated* *arrogates* *Seriate* *marches* *Interaction* *arrogates* **DISCUSSION** Several procedures were performed to confirm the identity of the unknown microorganism. Each test gave an indication to the microorganism's metabolic and physiological properties.

In this section, each procedure will be thoroughly discussed and each result explained. The Gram stain test is a differential stain for bacteria that identifies gram-positive bacteria from gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan cell membrane, while gram-negative bacteria have a thin peptidoglycan layer and an outer membrane. Gram-positive bacteria appear purple from the crystal violet retention. Gram-negative bacteria appear red from the loss of the crystal violet and the absorbency of the safranin counterstain. The unknown bacterium resulted in a red color under the oil immersion, indicating that the bacterium is gram-negative.

In addition, the cell morphology was also observed, and the bacterium was single bacillus. The next test performed was the aerodonetics test.

Aerodonetics is the ability of a microorganism to live or grow in the presence of oxygen. There are several categories that a microorganism can fall upon. In this test, there were only two options that could be observed: strict arroba and facultative anaerobe. A strict arroba lives and grows in the presence of oxygen, and the bacteria will grow near the top of the medium. A facultative anaerobe doesn't need oxygen to live, but can be used, and the bacterium will disperse throughout the broth medium. The result of this test was that the bacterium was a facultative anaerobe.

The methyl red test is used to identify enteric bacteria based on their pattern of glucose metabolism. The bacteria produce pyrrhic acid from glucose metabolism, and some cetera subsequently use the mixed acid pathway to metabolize pyrrhic acid to other acids. Bacteria that metabolize pyrrhic acid to other acids lower the pH of the medium, and turn the broth red, resulting in a positive test. Bacteria that metabolize pyrrhic acid to neutral end-products lower the pH of the medium, turning the broth yellow, resulting in a negative test. The bacterium in this test resulted in a yellow- orange color, making it MR. negative. The Dense test followed.

The purpose was to test for presence of the economy Dense which catalysts the hydrolysis of DNA. The ability to produce Dense can be determined by culturing a bacterium on the agar plate, which is composed of peptides, casein, sodium chloride, DNA and methyl green dye. After incubation, clearing on the agar around the bacterium indicates the presence of Dense, which is considered a positive response. This test did yield a positive result.

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SIMI medium tests for three activities that a bacterium can perform: sulfur reduction, indole production from tryptophan, and motility. For the purpose of this unknown project, the emphasis was on sulfur reduction and indole production.

Sulfur reduction results in the production of hydrogen sulfide, which reacts with the ampoule sulfate to form iron sulfide precipitate. In ten minutes, a black precipitate is an indication of sulfur reduction, and is considered a positive result. This test was negative, because there was no black precipitate in the medium. Indole production occurs in the presence of tryptophan. Bacteria that contain transaminases will hydrolyze the tryptophan to pyruvate, ammonia and indole. After incubation, Kovacs' reagent is added. The reagent contains dimethylaminobenzaldehyde, which will react with indole, and turn the reagent layer red, yielding a positive result. The unknown bacterium tested negative for indole production. Next was the test for lysine decarboxylation.

The medium for this test contained peptone, glucose, potassium phosphate and pyridoxal phosphate. After inoculation, mineral oil is added to promote glucose fermentation. If glucose fermentation occurs, the medium will turn yellow due to the accumulation of acidic products. Decarboxylation results in the accumulation of alkaline end products which turn the medium purple. There will be no color change if there is no fermentation and no decarboxylation. In this test, the broth turned purple. There was fermentation of glucose and decarboxylation. The Simmons citrate test is used to determine the ability of a microorganism to use citrate as its sole source of carbon.

The medium contains sodium citrate, ammonium dehydrogenate phosphate, and brotherly blue. Citrate-positive bacteria will produce ammonia and ammonium hydroxide, which turns the slant blue. The blue color is a positive test result. In this test, the slant was blue, and there was growth around the inoculation indicating a positive result. Purple Broth tests for fermentation abilities. The medium consists of carbohydrates, potent and processes purple. If the pH is lowered and fermentation occurs, the broth will turn yellow. If the pH is raised and fermentation does not occur, the broth will turn purple. Additionally, the presence of an inverted Durham tube tested for gas production.

If bubbles appear in the tube after incubation, gas production occurred. The result of this test indicated that the bacterium did undergo fermentation given the yellow broth, and that it produced gas due to the bubbles in the tube. The Triple Sugar Iron test fraternities bacteria on the basis of glucose fermentation, lactose fermentation, sucrose fermentation, and sulfur reduction. The medium contains proteins, ferrous sulfate, sodium tressellate and phenol red. When the agar is inoculated with a glucose-only fermented, acidic products lower the pH and turn the medium turns yellow. As the glucose is used up, amino acids break down, raise the pH and turn the medium red.

The result for a glucose-only fermented is a yellow butt and red slant. Bacteria that ferment both glucose and either lactose or sucrose give a result in a yellow butt and yellow slant. Bacteria that don't ferment glucose, lactose or sucrose result in a red butt and red slant. For this test, the medium had a yellow butt and red slant. The agar had no black precipitate,

yielding a negative result for sulfur reduction. There were large cracks in the agar, indicating gas production. This confirms the result from the purple broth test. The final test for the unknown was the urea test. Urea is a product of decarboxylation, and it can be hydrolyzed to ammonia and carbon dioxide by bacteria containing urease.