

Microbiology lab report assignment



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They are as follows: lactose fermentation on eosin methyl blue (EMBED), ITS (Triple Sugar Iron agar), Phenol red sucrose, the SIMI test, HAS by SIMI, Movie (indolent, motility, vogues-prosperous, ND citrate), Areas (urea broth), Updates (Phenylalanine Dominate), Lysine Destroyable, and Ernestine Destroyable. Colonial morphology on EMBED was used to prove the identity of the bacteria. The unknown bacteria was #9. After performing the tests it is determined that the unknown bacteria is Escherichia coli. 3. Methods: All tests were performed using the aseptic technique to assure the cultures were pure and not contamination was present.

The inoculation loops and straight wire stab needles were placed over the flame of a Bunsen burner and heated until red hot (sterile) then cooled before placing them into he bacteria to prevent the killing of the bacteria. The open end of each tube of bacteria was flamed once it was open (before inoculation) and before it was closed (after inoculation). (Morel, 9th Edition. 2008) EMBED (eosin methyl blue) agar was used to determine if the bacteria was a lactose ferment or a non- lactose ferment. EMBED agar is a selective and differential media.

The dyes eosin Y and methyl blue found in the medium inhibit the growth of gram-positive bacteria but not the growth of gram- negatives. Lactose ferments metabolize the lactose in the media and produce acid byproducts, causing a color change in the colony which is dark purple, almost black. Strong acid productions by organisms result in a metallic green sheen. Weaker fermentation of lactose results in colonies with a pinkish-purple color. Colonies that are non-lactose ferments remain colorless, or at least no darker than the color of the media.

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The unknown bacteria #9 was streak diluted on to an EMBED agar plate using a sterile inoculating loop. After incubation, the unknown bacteria #9 produced dark purple/black colonies that had a green metallic sheen. This is a typical test result from the bacteria *Escherichia coli*. Phenol Red Sucrose broth is a general-purpose differential test medium typically used to differentiate gram negative enteric bacteria. It contains phenol red (a pH indicator), a Durham tube, and one carbohydrate (sucrose). Phenol red is a pH indicator which turns yellow below a pH of 6.8 and fuchsia above a pH of 7.4.

If the organism is able to utilize the carbohydrate, an acid by-product is created, which turns the media yellow. If the organism is unable to utilize the carbohydrate but does use the protein, the by-product is ammonia, which raises the pH of the media and turns it fuchsia. When the organism is able to use the carbohydrate, a gas by-product may be produced. If it is, an air bubble will be trapped inside the Durham tube. If the organism is unable to utilize the carbohydrate, gas will not be produced, and no air bubble will be formed. The unknown bacteria #9 was added to the phenol red sucrose broth using a sterile inoculation loop.

Only a loop full was added and mixed into the broth. After incubating, the unknown bacteria #9 caused the broth to change color from red to yellow indicating a positive result for sucrose. There were bubbles inside the Durham tube which is a positive result for gas production. SIMI medium is a combination differential medium that tests three different parameters, which are represented by the three letters in the name: Sulfide-Indolent-Motility medium. It is a semi-solid agar that is <https://assignbuster.com/microbiology-lab-report-assignment-lab-report-samples-2/>

inoculated with a bacterium to test for hydrogen Sulfide, Indolent, and Motility of the organism.

The medium is inoculated by a stab method (stab a straight hole through the medium using a straight wire with the bacteria on it). Incubate the bacteria for about 24 hours and then begin testing. If hydrogen sulfide is present, it will react with the sodium tressellate in the medium and the indicator, Eric ammonium citrate, to produce ferrous sulfide which falls out of solution as a blackish precipitate. The presence of hydrogen sulfide typically means that the bacteria produces the enzyme cytosine desalinate which breaks up the cytosine in the medium into hydrogen sulfide. A positive result will turn the medium black.

If the result is negative then the medium will stay semi-clear. The unknown bacteria #9 produced negative results for HAS in the SIMI test tube. The medium stayed semi-clear and did not turn black. Movie reactions are a set of four reactions that are: Indolent test, Methyl Red test, Vogues Prosperous test and Citrate utilization test. The letter “ i” is only for rhyming purpose. The Indolent portion of the test is performed by adding Kava’s reagent to the inoculated SIMI medium. The Kava’s reagent reacts with the indolent (if indolent is present) to produce a pinkish-red or reddish-purple ring around the top of the test tube.

If indolent isn’t present, there will be no color change. The presence of indolent indicates that the bacteria produces transparency, an enzyme which breaks down thyrotrophic into smaller components, one of which being indolent. The results for the indolent test for unknown bacteria #9 were

positive. When the Kava's reagent was added to the SIMI tube, the top of the medium turned a ruby red color which indicates a positive test result. Methyl Red (MR..) and Vogues-Prosperous (UP) broth is used as a part of the Movie tests as the medium in which both the Methyl Red and Eavesdropper's tests can be performed.

It is a simple broth that contains potent, buffers, and dextrose or glucose. To perform these tests a loop full of the bacteria is added to the broth and incubated. Methyl red is a dye that is acid-sensitive. It turns yellow at a pH above 4.5 and when the pH is below 4.5 it turns red. The Methyl Red test involves adding the pH indicator methyl red to an inoculated tube of MR..-UP broth. If the organism uses the mixed acid fermentation pathway and produces stable acidic end-products, the acids will overcome the buffers in the medium and produce an acidic environment in the medium.

When methyl red is added, if acidic end products are present, the methyl red will stay red. The UP test detects organisms that utilize the butyrate glycol pathway and produce action. When the UP reagents are added to MR..-UP broth that has been inoculated with an organism that uses the butyrate glycol pathway, the action ND product is oxidized in the presence of potassium hydroxide (KOCH) to dedicate. Creating is also present in the reagent as a catalyst. Dedicate then reacts to produce a red color. Therefore, red is a positive result.

If, after the reagents have been added, a copper color is present, the result is negative. Neither of these tests were performed during this experiment.

The test results were given to the students by the teacher. When methyl red

is added to MR..-UP broth that has been inoculated with *Escherichia coli* (#9), it stays red. This is a positive result for the MR.. Test. When the UP reagents are added to MR..-UP broth that has been inoculated with *Escherichia coli* (#9), the media turns a copper color. This is a negative result for the UP test. Simmons citrate agar tests the ability of organisms to utilize citrate as a carbon source.

Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dehydrogenate phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromothymol blue. This test is part of the Movie tests. Organisms which can utilize citrate as their sole carbon source use the enzyme citrate permease to transport the citrate into the cell. These organisms also convert the ammonium dehydrogenate phosphate to ammonia and ammonium hydroxide, which creates an alkaline environment in the medium. At pH 7.5 or above, bromothymol blue turns royal blue.

At a neutral pH, bromothymol blue is green, as evidenced by the inoculated media. If the medium's slant turns sapphire blue, the organism is citrate positive. If there is no color change, the organism is citrate negative. A loop full of the unknown bacteria #9 was inoculated onto the citrate slant and incubated. There was no change in the green color of the agar which means the results for citrate for #9 were negative. Areas broth is a differential medium that tests the ability of an organism to produce an exogenous, called urease that hydrolyzes urea to ammonia and carbon dioxide.

The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Phenol red turns yellow in an

acidic environment and fuchsia in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink. Phenylalanine dominate (Updates) medium tests the ability of an organism to produce the enzyme phenylalanine ammonia-lyase. This enzyme removes the amine group from the amino acid phenylalanine and releases the amine group as free ammonia. As result of this reaction, phenylalanine acid is also produced.

After incubation, 10% ferric chloride is added to the media; if phenylalanine acid was produced, it will react with the ferric chloride and turn the slant a bluegreen color. Fifth medium remains a straw color, the organism is negative for phenylalanine dominate production. A loop full of unknown #9 was added to the slant of the Updates test tube. After incubation, five drops of 10% ferric chloride was added to the slant. There was no reaction so the results are negative. Lysine Destroyable (OLD) is an amino acid that possibly can be broken down by the destroyable enzymes that are in some bacteria.

The carbonyl (COHO) group on the amino acid molecule is removed during this process. As a result, alkaline end products that change the pH indicator color are left. The pH indicators are broodmares purple and cresol red.

Broodmares purple turns purple at an alkaline pH and turns yellow at an acidic PH. The experiment works best when air is excluded from the test tube, so a layer of mineral oil is added after a pop full of the bacteria is inoculated and before incubation. The results for the unknown bacteria #9 were positive. The medium turned purple indicating a positive result.

A negative result would have been if the medium turned yellow. Colonial morphology was the determining test to prove that unknown bacteria #9 was indeed *Escherichia coli*. The dark purple colonies with a green metallic sheen of the unknown #9 was the same as *Escherichia coli*. Entertainer agglomerate (the only other bacteria left) colonies have a dark purple center surrounded by a wide, light-colored purple, mucous rim – resulting in a “fish-eye” yep of colony. 4. Results: The indolent test had an unusual result. The first time the test was performed the Kava’s reagent was added and it turned an orange color.

After allowing the test tube to sit for a few days, the agar medium turned an orange color. The test was repeated and the results from this second test confirmed that the unknown bacteria #9 was positive for indolent. After adding the Kava’s reagent, it turned a ruby red color indicating a positive result. Another test that had an unusual result was the phenol red sucrose broth. The tube was inoculated and left to incubate. The original color of the broth was red. After incubation the broth had changed to orange, which was a weak reaction.

After letting the test tube incubate for a few more days, the broth had changed to yellow and bubbles were in the Durham tube. This indicated a positive result for both sucrose and gas production. *see attached Bacteria and Tests Performed Chart *see attached Flow Chart 5. Discussion of the Medical or Ecological Significance: The genus *Escherichia* is named after Theodore Escherichia, who isolated the type species of the genus.

Escherichia organisms are gram-negative bacilli that exist singly or in pairs.

Escherichia coli is a facultative anaerobic with a type of metabolism that is both fermentation and respiratory.

They are either non-motile or motile by perspicuous flagella. Escherichia coli is a major facultative inhabitant of the large intestine. Escherichia coli of many different grottoes, are categorized into four major groups according to virulence mechanisms: anthropogenic (ETC); anthropogenic (OPEC); interpretative (ICE); and interrogatives (Gage SEC). Other groups (e. G. , diffusely adherent E. Coli) are less well established as pathogens. Escherichia coli is a bacterium that is commonly found in the gut of humans and other warm-blooded animals. While most strains are harmless, some can cause severe food borne disease.

Escherichia coli infection is usually transmitted through consumption of contaminated water or food, such as undercooked meat products and raw milk. Symptoms of disease include abdominal cramps and diarrhea, which may be bloody. Fever and vomiting may also occur. Most patients recover within 10 days, although in a few cases the disease may become life-threatening. Escherichia coli is also one of the most frequent causes of many common actuarial infections, including collectivities, bacteria, echolocation, urinary tract infection (OUT), and traveler’s diarrhea, and other clinical infections such as neonatal meningitis and pneumonia.

Most Escherichia coli infections can clear up on their own after a few days. The Escherichia coli 0157: HE infection is well known for causing not only severe diarrhea, but also possible life threatening complications mostly in children and the elderly. Some of the complications are as follows: renal

failure, anemia, and dehydration especially for children (termed HUSH or Hemolytic-uremia syndrome) ND spontaneous bleeding, organ failures, and mental changes in the elderly (termed HTTP or thrombosis thermodynamic purport).