Short essay about lab report

Business

Microbiology Laboratory Report Identification of Unknown Bacteria 03/10/05-04/01/05 Authors: Richard Hendricks, Jessica Prebish; NMU Abstract: Broth culture 16 was randomly selected by our group and subjected to qualitative tests for taxonomic identification.

The culture did appear homogenous throughout the testing period and is currently retained by Northern Michigan University's department of Microbiology. We suggest that culture 16 is an example of Escherichia coli. Background: Techniques used were in accordance with NMU Professor Dr. D. Becker's lab manual (ISBN 0-390-53911- 2; McGraw Hill). Changes in protocol or interpretation are noted where they were implemented, but strict adherence to the manual prevailed.

Materials and Methods: Microscope, incubator, and deionizer functioned correctly throughout testing period, with stains, dishes, agars, and test reagents readily available. Lab procedures are considered orthodox and usage thereof is noted chronologically. 3/15/05 We obtained a 24 hour old stock broth culture of the unknown specimen 16. It appeared turbid, reflecting a substantial amount of growth. To determine that the stock culture was pure, we performed a streak plate using loops of the stock broth on a fresh dish of nutrient agar and then incubated it for 48 hours at Standard Temperature and Pressure; 37C, 1 ATM (STP).

A Gram stain was then carried out to differentiate the unknown sample from a broad class to a more specific category of bacteria. The Gram stain distinguishes between Gram-positive and Gram-negative bacteria based on the composition of the cell walls. Gram-positive bacteria appear purple and

Gramnegative bacteria appear pink after staining. The first Gram stain produced unsatisfactory results and was then repeated with a clear indication of negativity. Light pink staining was evident on the cells in the field of view (FOV) and a search of the slide revealed uniformity in the sample.

From these results we concluded that unknown 16 was Gram-negative in nature, and therefore it could possibly be any of the following bacteria: Alcaligenes faecalis, Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris, or Pseudomonas fluorescens. We then conducted a negative stain to reveal the shape of the cell and any extracellular features such as the presence of a capsule. The first negative stain was unsuccessful and was therefore repeated. The second stain yielded cocci and coccobacilli shaped cells occurring singly and also arranged in irregular clusters. These appeared somewhat sparsely distributed at 1000x with oil immersion.

A simple stain was then performed because previously in lab, the negative stain made it hard to visualize the bacteria. The simple stain was prepared using a sample of bacteria from the 24 hour old broth culture. The appearance of the microbe was again coccus and coccobacillus in shape, yet clusters were grouped in greater numbers, and their concentration was higher for the same FOV. From the results obtained by the negative and simple stain, we assumed that the unknown sample might be Alcaligenes faecalis, due to the shape of the cells and their clustered pattern. 3/17/05 After 48 hours of incubation, the results of the streak plate were particularly successful. This revealed moist, colorless, and opaque streaks of colonies the

size of pinpricks in quadrant one, diminishing to one 3 mm round colony in the fourth quadrant that had billowing or irregular edges.

All colonies appeared to be the same color, size, and shape. We concluded that the stock broth culture was pure. A Potassium Hydroxyl (KOH) Gram test was then performed to confirm the results of our Gram stain and to assure that our bacteria was Gram negative. The KOH test revealed an extremely fine filament which adhered to a toothpick and stretched three to five mm before breaking from tension. This was first performed with a single loop from the streak plate prepared on 3/15/05 with positive results obtained, and repeated with a more generous smear of five loops. The larger smear of bacteria when mixed with KOH turned nearly opaque and produced a more prominent filament that again retained adhesion to the toothpick for three to five mm before breaking.

Based on the results of the Gram stain and the KOH Gram test we concluded that our unknown sample was a Gram-negative. To determine if our unknown sample was Alcaligenes faecalis, we decided to perform an oxidase test. Some gram-negative species of bacteria are oxidase positive while others, such as those in the Enterobacteriaceae family, are oxidase negative, so we prepared a tryptic soy agar plate and made a single streak-line inoculation using the 72 hour old stock broth culture. We incubated this plate for 24 hours. 3/18/05 After the tryptic soy agar plate had incubated, we applied a KEY oxidase Test Strip to the surface of the agar plate, covering the colony formed from the streak-line inoculation. The KEY oxidase Test Strip did not show color change from pink to dark purple.

This test revealed that the unknown sample was oxidasenegative and therefore unlikely to be Alcaligenes faecalis. This led us to believe that our unknown may be Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, or Proteus vulgaris. See figure 2. 3/22/05 To differentiate between Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, and Proteus vulgaris we decided to test carbohydrate fermentation pathways of the unknown sample. We inoculated three Durham tubes containing glucose, sucrose, and lactose, with a loop of our stock broth culture that was 192 hours old and incubated the tubes for 48 hours. We then prepared a TGYA shake tube to determine the unknown sample’s oxygen requirements.

We inoculated the TGYA shake tube with the stock broth culture and incubated this for 48 hours. 3/24/05 The results of the TGYA shake tube showed consistent growth of the unknown specimen throughout the tube. We concluded that the bacterial sample was a facultative anaerobe because it grew at the bottom of the shake tube in the absence of oxygen and also toward the top of the tube where oxygen was present. These results further supported our claim that our unknown was not Alcaligenes faecalis because it is an obligate aerobe and also it eliminated the possibility of our unknown being Pseudomonas fluorescens because it too is an obligate aerobe. The results of our Durham tube test showed that the unknown sample was able to utilize various carbohydrate fermentation pathways. All three test tubes containing Glucose, Sucrose, and Lactose were positive for the presence of acid and gas production.

A positive result was determined by the color change of the medium from red to yellow and the presence of a gas bubble within the Durham tube.

From these results we concluded that our unknown sample could not be Proteus mirabilis or Proteus vulgaris due to the fact that neither of these bacteria is able to ferment lactose. We determined that the broth culture specimen was either Escherichia coli or Enterobacter aerogenes because they both are facultative anaerobes that are able to ferment Glucose, Sucrose, and Lactose. To differentiate between Escherichia coli and Enterobacter aerogenes we decided to run the IMViC tests. For the indole production test, we inoculated a SIM deep tube with a loop of stock broth culture that was 240 hours old and incubated it for 24 hours. For the Methyl Red and Voges-Proskauer Test, we inoculated a MR-VP broth media tube with the stock broth culture and again incubated it for 24 hours.

For the Citrate Utilization Test, we inoculated a Simmons citrate agar slant using a stab-and-streak method and incubated the tube for 24 hours. 3/25/05After completing the indole production test, no color change from colorless or light yellow to deep red was observed therefore a negative result was obtained. As for the Methyl Red Test, a positive result was recorded because a red color change occurred. The Voges-Proskauer test revealed a negative reaction because no color change occurred and also the citrate utilization test was negative because the tube remained green in color and did not turn blue. From the results of the IMViC Tests we concluded that unknown specimen 16 was Escherichia coli. From previous tests conducted in lab, it was known that Enterobacter aerogenes will produce a negative methyl red test, a positive Voges-Proskauer test, and a positive citrate utilization test.

Just the opposite result is true for Escherichia coli and our IMViC tests supported these findings. Overall the final conclusion that our unknown sample was in fact Escherichia coli was determined by examining all of our data from the Gram stain, KOH test, oxidase test, oxygen requirement test, carbohydrate fermentation, and the IMViC tests to further support our claim. Acknowledgements: Dr. s Becker, Lopez, Rebers, and their staffs facilitated our efforts with forbearance and trust, their approval and understanding of lab time overruns made our work more precise. Thanks are owed to them in superscript.

References: Benson-Brown, Amy (2001) Microbiological Applications Lab Manual; Complete version, 9e Harley, John (1996) Laboratory Exercises in Microbiology, Sixth Ed. J. G. Holt, Lippincott, Williams and Wilkins, 9th ed. (1994) Bergey’s Manual of Determinative Bacteriology Kleyn, J.

Bicknell, M. (2001) Microbiology Experiments: A Health Science Perspective, 4e Table 1. Results for the Identification of Unknown 16 Gram stain – KOH Gram test – Colony formation moist, colorless, opaque, irregular edges Negative stain cocci, coccobacillus Simple stain cocci, coccobacillus TGYA shake tube facultative anaerobe Lactose + Acid, + Gas Sucrose + Acid, + Gas Glucose + Acid, + Gas Oxidase – Indole + Methyl red + Voges-Proskauer – Citrate – Figure 2. Oxidase test results