Microbiology: unknown organism - lab report example

Science, Biology

Microbiology: Unknown Organism

Microbiology Lab Report 23 February Unknown Organism Report Purpose To identify an unknown microorganism by performing a series of biochemical tests on a pure bacterial culture.

Materials and Methods

1. Carbohydrate Fermentation: Two culture tubes containing sucrose broth and lactose broth were inoculated with a pure culture of the unknown microorganism and incubated at 37oC. Each broth contained phenol red, a pH indicator that changes from red in basic environment to yellow in acidic conditions. The color change was an essential indicator of acid formation from the fermentation of the sugars during the growth of bacteria. An inverted Durham tube filled with the broth was also included in the setup to trap any gas released during fermentation.

2. Casein hydrolysis: A skim milk agar plate was inoculated with the unknown microorganism in a straight line. The plate was incubated at 37oC. The area around the microorganism was observed for clarity, which was a positive indicator for the ability of the microorganism to hydrolyze casein.

3. Catalase activity: A nutrient agar plate was inoculated with the unknown microorganism by streaking. The plate was overturned and incubated at 37oC. 3% hydrogen peroxide was dropped onto an isolated colony of the microorganism and the plate observed for bubbles. The formation of bubbles was an indication of the breakdown of hydrogen peroxide.

4. Sulfide production: A tube containing the sulfite indole motility (SIM) medium was inoculated with the unknown microorganism. The tube was then incubated at 37oC. The medium was observed for the formation of a black

precipitate, which was a positive indicator for the production of sulfide.

5. Starch hydrolysis: A starch agar plate containing nutrient agar and starch was inoculated with the unknown microorganism and incubated for some time at 37oC. The plate was then flooded with iodine and observed for color change. A color change from straw-colored to blue around the growth of the microorganism was an indicator of the inability of the microorganism to hydrolyze starch. A clear color, on the other hand, showed the ability of the microorganism to break down starch.

6. Tryptophan hydrolysis: The unknown microorganism was inoculated into a tube containing 1% tryptone broth and incubated at 37oC. 5 drops of Kovac's reagent were added to the tube, which was shaken gently and allowed to stand for about 10 minutes. The appearance of a red layer at the top of the reagent tube indicated the presence of indole, whereas its absence indicated the absence of indole in the medium.

7. Urea hydrolysis: The assigned microorganism was inoculated into a tube containing urea broth. A color change of the PR indicator from orange to pink indicated the presence of urea.

Results

1. Carbohydrate fermentation: The growth of the unknown organism caused the PR to turn from red to yellow in the sucrose medium with the production of gas in the Durham tube. However, there was no color change or gas production in the lactose broth.

2. Casein hydrolysis: The area around the unknown microorganism inoculum was white (not clear).

3. Catalase activity: There was the formation of gas bubbles around the

colony on addition of 3% hydrogen peroxide.

4. Sulfide production: There was the formation of a black precipitate in the tube containing the SIM medium and the bacteria because of the growth of the unknown microorganism.

5. Starch hydrolysis: The area around the unknown bacterial growth was clear.

6. Tryptophan hydrolysis: There was the formation of a red ring on top of the medium.

7. Urea hydrolysis: The PR indicator in the urea broth changed from orange to pink.

Table of Metabolic Activities

Discussion

The unknown microorganism was able to ferment sucrose anaerobically hence the production of carbon dioxide and the color change of phenol red indicator from red to yellow. However, the microorganism was not able to ferment lactose. The area around the microorganism in the skim milk agar plate remained white indicating that the unknown microorganism did not hydrolyze casein. The addition of hydrogen peroxide to a colony of the microorganism yielded bubbles indicating that the bacteria produced the enzyme catalase, which was accountable for the formation of bubbles as it decomposed hydrogen peroxide into water and oxygen gas (Winn & Koneman 38).

The formation of a black precipitate in the SIM medium showed that the unknown microorganism produced hydrogen sulfide through the decomposition of sulfur-containing amino acids by the bacteria’s enzymes.

The SIM test also indicated the motility of the organism (“ Proteus vulgaris, a Motile Organism, in a Sulfide Indole Motility Deep”). The area around the unknown bacteria in the starch agar medium was clear after the addition of iodine. This revealed that the unknown microorganism produced the enzyme amylase, which was responsible for the hydrolysis of starch into single units of glucose.

A red ring was formed on top of the reagent tube containing tryptone medium. This was an indication of the production of the enzyme tryptophanase by the unknown microorganism. Tryptophanase was responsible for the breakdown of tryptophan into indole and pyruvic acid. The urea broth changed to pink because the unknown bacteria produced urease that decomposed urea into ammonia and carbon dioxide.

Conclusion

From the obtained results, the identity of the unknown microorganism was the bacterium Proteus vulgaris. However, the test results did not perfectly match those of Proteus vulgaris on the chart. The differences in the casein hydrolysis test were probably due to due to faults in culture preparation (such as contamination of the culture with other microorganisms), skim milk agar preparation or in the course of the experimental process. The differences between unknown and all other bacteria were greater than the differences between the unknown microorganism and Proteus vulgaris. In addition, the observed traits (hydrolysis of starch, casein, and production of ammonia, hydrogen sulfide and indols) match those of documented traits of Proteus vulgaris (Herter and Broeck 497).

Works Cited

Herter, C. A. and Broeck, C. T. “ A Biochemical Study of Proteus vulgaris Hausa.” The Journal of Biological Chemistry. 1911. 9 (1911): 491-511. Web. Feb. 23 2013.

“ Proteus vulgaris, a Motile Organism, in a Sulfide Indole Motility Deep” n. d. Web. 23 Feb. 2013 .

Winn, W. C. and Koneman, E. W. Konemans Color Atlas and Textbook of Diagnostic Microbiology, Philadelphia, PA: Lippincott Williams & Wilkins, 2006. Print.