**Microbiology**

Your Name

Department, University

Course number: Course name

Professor’s Name

Date

**Unknown Identification**

1. **Gram Stain**

**Purpose:** Gram stain is used to differentiate the gram-positive and gram-negative bacteria based on their response to the dyeing/staining of the peptidoglycan cell wall (Finazo & Obenauf, 2018, p.68).

**Result:** After the staining process, the gram-positive bacteria will appear purple, while gram-negative bacterial cells will appear pink (Finazo & Obenauf, 2018, p. 71). The technique decolorizes the thick layer of peptidoglycan in the cell wall of the gram-positive bacteria and traps the crystal violet dye complexes during the gram staining. The dye crystals remain in the cell wall after the application of iodine and alcohol, thus making the gram-positive bacteria purple throughout the gram staining process. On the gram-negative bacteria, the cells would not retain the crystal violet dye in their thin peptidoglycan cell wall. However, since they appear colorless after the application of alcohol, the red dye safranin will counterstain their cell walls to appear pink (Finazo & Obenauf, 2018, p. 69).

1. **IMViC Reactions**

IMViC Reactions are a group of different biochemical test that stands for indole, methyl red, Voge-Proaskauer, and citrate (Finazo & Obenauf, 2018, p. 166).

* 1. **Indole**

**Purpose:** Indole test uses the SIM medium with Kovac's reagent to test for the production of indole from the enzymatic hydrolysis of tryptophan in the sample. SIM is an acronym, which stands for sulfide, indole, and motility that are used to test the different characteristics of the organism with a single stab inoculation (Finazo & Obenauf, 2018, p. 166).

**Results:** In hydrogen sulfide production, the formation of a black precipitate indicates H2S positive reaction (Finazo & Obenauf, 2018, p. 166). The SIM medium contains iron salt and thiosulfate that reacts with hydrogen sulfate produced by the organism to produce a black precipitate (Finazo & Obenauf, 2018, p. 168).

In the indole production, a cherry-red colored surface on the agar tube indicates an indole positive reaction, while no change in color that remains pale yellow indicates an indole-negative reaction (Finazo & Obenauf, 2018, p. 167). The cherry-red by-product is produced by organisms that secrete tryptophanase that hydrolyzes tryptophan to produce indole, pyruvic acid, and ammonia (Finazo & Obenauf, 2018, p. 168).

In Motility, the tube will turn cloudy or turbid. The turbidity of the tube indicates the movement of the organisms away from the line of inoculation (Finazo & Obenauf, 2018, p. 168).

* 1. **Methyl Red**

**Purpose:** The methyl red tests indicate whether the end products of carbohydrate metabolism of the organisms have acidic pH (Finazo & Obenauf, 2018, p. 170).

**Results:** If the medium turns red, it indicates a positive result that the carbohydrate fermentation produced an acidic end product with a pH value of less than 4.4. If the medium did not change in color, it indicates a negative result that the carbohydrate fermentation produced an end product with a pH value of 6.2 or higher (Finazo & Obenauf, 2018, p. 170).

* 1. **Vogues-Proskauer**

**Purpose:** The Voges-Proskauer tests indicate whether the end products of carbohydrate metabolism of the organisms have neutral pH (Finazo & Obenauf, 2018, p. 170).

**Results:** If the medium turns brownish-red, it indicates a positive result that the carbohydrate fermentation produced an end product with neutral pH. If the medium did not change in color, it indicates a negative result that the carbohydrate fermentation produced an end product with acidic pH (Finazo & Obenauf, 2018, p. 170).

* 1. **Citrate**

**Purpose:** The citrate test is used to observe the ability of the organism to use citrate as the only source of carbon in the medium and indicate the increase of alkalinity or pH (Finazo & Obenauf, 2018, p. 171).

**Results:** If the medium turns from green to bright blue or Prussian blue, it indicates a positive result. Ammonia is released in the reaction that increases the pH of the medium. The Bromothymol blue reagent indicates the increase of alkalinity that is green when in neutral pH but turns bright blue in high pH levels (Finazo & Obenauf, 2018, p. 171).

1. **Starch Hydrolysis**

**Purpose:** Starch Hydrolysis is used to determine the presence of organisms that can produce amylase that can catalyze the hydrolysis of amylose and amylopectin to a limited extent (Finazo & Obenauf, 2018, p. 147).

**Results:** Starch turns blue-black when iodine is added (Finazo & Obenauf, 2018, p. 149). Therefore, a positive result will show that the agar surrounding the colony will be colorless upon the addition of iodine since the production of extracellular amylase hydrolyzed the starch in the agar. On the other hand, a negative result will show that the agar surrounding the colony will turn blue-black upon the addition of iodine due to the presence of starch in the agar (Finazo & Obenauf, 2018, p. 150).

1. **Catalase Test**

**Purpose:** The catalase test is used to determine the organisms that can produce enzyme catalase to convert hydrogen peroxide to water and oxygen gas (Finazo & Obenauf, 2018, p. 119).

**Results:** Bubbles appear on the colony that is positive for the catalase test. Bubbling indicates the breakdown of H2O2 and the production of O2 gas. No bubbling indicates negative results, which shows that the organism does not produce catalase (Finazo & Obenauf, 2018, p. 121).

1. **Oxidase Test**

**Purpose:** The oxidase test is used to determine if the bacteria contain cytochrome c oxidase enzyme that reduces oxygen gas to form water (Finazo & Obenauf, 2018, p. 127).

**Results:** A positive result will turn the sample to dark blue or purple while a negative result will be colorless. The dark blue or purple color indicates the reduction reaction between the sample and the oxidase reagent. The colorless result indicates the oxidation reaction between the sample and the oxidase reagent (Finazo & Obenauf, 2018, p. 127).

1. **Nitrate Reduction**

**Purpose:** The nitrate reduction test is used to determine the production of nitrate and nitrite in the inoculated medium (Finazo & Obenauf, 2018, p. 186).

**Result:** After the incubation, red coloration in the tube results from the addition of reagents A and B indicates a positive result for the presence of nitrite. Reagent A and B when nitrite is present and occurrence of nitrate reduction. However, a colorless result in the tube indicates the presence of nitrate or nitrate was reduced to nitrous oxide (Finazo & Obenauf, 2018, p. 189).

In the next part of the procedure, zinc and HCl were added to the colorless tube. A colorless reaction indicates the positive result in the presence of nitrous oxides or nitrogen gas, which indicates nitrate and nitrite reduction (Finazo & Obenauf, 2018, p. 189). On the other hand, a change in red coloration indicates the presence of nitrate, which is a negative result. Zinc with HCl catalyzes the conversion of nitrate to nitrite; however, since reagents A and B are present in the tube, the tube will turn red, which implies a negative result for the reduction of nitrate (Finazo & Obenauf, 2018, p. 187).

1. **Identification**

Organism #2 is *Escherichia coli.* Organism #2 is gram-stain negative, where pink-colored rods are observed. The catalase test indicates a positive result, where bubbles appeared on the slide. The oxidase test is negative, where no color change was observed. The indole test shows a positive result with the formation of a cherry-red color on the top of the media. The methyl red test is positive that turned the color of the broth to red. The Voges-Proskauer test is negative, which shows no change in coloration. The citrate test is also negative, and the color of the slant remains green. The H2S test is also negative since not black precipitate formed in the media. The starch hydrolysis test is negative since the plate remained blue-black color throughout. Lastly, the nitrate reduction test shows a positive result in the presence of nitrate upon the addition of reagents A and B that immediately showed a red color. All of the presented characteristics indicate that the unknown organism is *E. coli.*

Escherichia coli are normally found on the intestinal systems of mammals that assist their host in breaking down carbon compounds in the body. Researchers often use E. coli as an indicator of fecal contamination in the area, especially in the waterways. However, *E. coli* can also be naturalized in any nutrient-rich area in tropical and temperate environments (Ishii & Sadowsky, 2008). Many E. coli strains can be found in founding worldwide, especially in places where the areas are overcrowded and the sanitation is poor, usually in the soil, manure, irrigation water, and freshwater (Chekabab et al., 2013). Traditionally, E. coli are harmless to its host; however, certain strains are pathogenic to their hosts (Van Elsas et al., 2010, p. 173). The diseases usually associated with pathogenic strains include mild to bloody diarrhea that eventually culminates in hemolytic-uremic syndrome, which is characterized by blood disorders and kidney problems. Additionally, E. coli is found to be associated with urinary tract infections (Van Elsas et al., 2010, p. 174).

**References**

Chekabab, S. M., Paquin-Veillette, J., Dozois, C. M., & Harel, J. (2013). The ecological habitat and transmission ofEscherichia coliO157:H7. FEMS Microbiology Letters, 341(1), 1–12. <https://doi.org/10.1111/1574-6968.12078>

Finazo, S. & Obenauf, S. (2018). Laboratory manual for microbiology fundamentals: A clinical approach (3rd ed.). McGraw-Hill Education.

Ishii, S., & Sadowsky, M. J. (2008). Escherichia coli in the Environment: Implications for Water Quality and Human Health. Microbes and Environments, 23(2), 101–108. <https://doi.org/10.1264/jsme2.23.101>

Van Elsas, J. D., Semenov, A. V., Costa, R., & Trevors, J. T. (2010). Survival of Escherichia coli in the environment: fundamental and public health aspects. *The ISME Journal*, 5(2), 173–183. <https://doi.org/10.1038/ismej.2010.80>