

Quantification of microbes in water | experiment



**ASSIGN
BUSTER**

Abstract

Coliforms present in water sources are due to fecal contamination indicating mammal feces also indicating intestinal pathogens in the water source. The presence of coliforms - bacteria commonly found in the intestines of mammals - are most common indicators of the quality of water sources, as well as measuring the severity of coliforms present within the water sources. Within this laboratory experiment, multiple water reservoirs will be tested to examine the presence of coliforms ruling out those that are not commonly associated with human feces resulting in harmful pathogens. This report examines and evaluates the presence of coliforms in various water samples in the local Hattiesburg area - Lake Byron, Professor Littlejohn's Lake, water from a Dog fountain, and distilled drinking water. If there is any fecal contamination within our selected water sample, Lake Byron, then the sample will test positive for lactose and differentiate the strains of enteric bacteria showing that it is in fact associated with fecal contamination. After completing both the lactose and IMViC test, Lake Byron resulted in testing positive for lactose fermentation resulting in the production of both acid and gas meaning that there were harmful pathogens of fecal contamination detected within this particular water sample - proving my hypothesis to be correct.

Introduction

The presence of coliforms - bacteria commonly found in the intestines of vertebrates - are the most common indicators of organisms of the Enterobacteriaceae family, used to monitor water quality. (Littlejohn, 2017).

Coliforms are commonly useful in assessing both the presence of coliforms in areas of water as well as the safety of drinking water; however, the presence of coliforms in drinking water indicates pathogens that could be in the water system, which will be evaluated within this laboratory report. Within this laboratory experiment, multiple water reservoirs will be tested to examine the presence of coliforms ruling out those that are not commonly associated with human feces resulting in harmful pathogens. These harmful pathogens can lead to potential water-borne diseases being the cause of many outbreaks. (Pandey, Kass, Soupir, Biswas, & Singh, 2014).

This report examines and evaluates the presence of coliforms in various water samples in the local Hattiesburg area - Lake Byron, Professor Littlejohn's Lake, water from a Dog fountain, and distilled drinking water. Confirmation of Enterobacteriaceae in a water system indicates fecal contamination in water sources, aiding in the restoration of drinking water and other substances. (Martin, Hsieh, Boor, & Weidmann, 2016). We examined various water samples to detect whether coliforms were present using the most probable number (MPN) test whereas the production of both acid and gas indicated the presence of coliforms. After identifying the various coliforms present in the water samples, we performed the IMViC - indole production, Methyl-red test, Voges-Proskauer test, and the citrate utilization - test to differentiate between the different members of enteric bacteria, Enterobacteriaceae. If there is any fecal contamination within our selected water sample, Lake Byron, then the sample will test positive for lactose and differentiate the strains of enteric bacteria showing that it is in fact associated with fecal contamination.

Materials and Procedures

First Lab Session Materials

Materials for the first lab session include one 10 mL pipette and pump (green), a 1.0 mL pipette and pump (blue), 5 double-strength lactose fermentation broths with Durham tube, 10 single-strength lactose fermentation broths with Durham tube, a water sample, and Levine's eosin methylene blue (EMB) agar plate.

Procedures

We arranged a test tube rack with a 5 single-strength and 10 double-strength lactose broths, labeling 5 of the single-strength tubes 1.0 mL and the other 5 0.1 mL, with our group information - group name, section number, and water sample. Next, we agitated our water sample, Lake Byron, and transferred 10 mL of water to each of the 5 double-strength lactose broths. 1.0 mL of the Lake Byron water sample was transferred to the single-strength lactose labeled 1.0 mL, and 0.1 mL of the Lake Byron water sample was transferred to the single-strength lactose labeled 0.1 mL - all tubes were incubated for 24 hours at approximately 37 degrees Celsius. 24 hours later, each tube was examined and the number of visible bubbles from each set was recorded - indicating gas production while coloration changes, from red to yellow, indicated acid production. Selecting one positive tube, we inoculated the EMB agar plate to be sure that the gas producing bacteria were coliforms - we incubated the plate for 24 hours at 37 degrees Celsius, then at room temperature until the upcoming lab meeting.

<https://assignbuster.com/quantification-of-microbes-in-water-experiment/>

Second Lab Session Materials

Materials for the second lab include one lactose fermentation broth with Durham tube and one TSA slant.

Procedures

We observed the EMB agar plates for colonies of *E. coli* - small colonies in a distinctive metallic green color while other bacteria, like *Enterobacter aerogenes*, produce larger colonies, lacking the metallic green color. We recorded the results. Next, we created a sub-culture inoculating the TSA slant using a coliform colony, inoculating one single-strength lactose broth - labeling both the tube and slant with our group name, section number, and the type of water sample. Lastly, we incubated both tubes at 37 degrees Celsius for 24 hours, then at room temperature until the next laboratory meeting.

Third Lab Session Materials

The materials for the third lab session include gram stain reagents, two MRVP broths, one Tryptone broth, and one Simmons citrate agar slant.

Procedures

We observed the lactose broth for acid and gas production and recorded the results. Using the TSA slant, a colony was selected, and we performed a gram stain then recorded the results. Next, we performed an IMViC to determine the different types of enteric bacteria - labelling one tryptone broth, one citrate slant, and two MRVP broths; one containing our Lake Byron

water sample, including our group information - group name, section number, MR, and the water sample tested). Each tube was inoculated, using the aseptic technique, with a confirmed coliform.

Fourth Lab Session Materials

The materials for the fourth lab session included an ampoule of VPA (alpha-naphthol) reagent, an ampoule of VPB (potassium hydroxide) reagent, and an ampoule of indole (Kovac's) reagent.

Procedures

MRVP media measures specific by-product productions of fermentative pathways - this requires two tests, one negative and one typically resulting as positive, with the addition of reagents. Five drops of methyl red were added to the culture while agitating the culture - red liquid change results in a positive reaction while no change results in a negative reaction, indicating butanediol production. Vogues-Proskauer test indicates 2, 3 butanediol production - we added one ampoule of VPA and VPB reagents, agitating the tube resulting in the 2, 3 butanediol being oxidized to acetoin - red coloration in approximately 30 minutes indicated a positive result for acetoin. We examined the citrate slant - if the organism lacked citrate, it remained green, if the organism contained citrate it turned bright blue. The tryptone broths were examined; aiding in the indication of the hydrolysis of tryptone into indole, pyruvate and ammonia by red ring formation at the top of the tube. Negative test result in either beige or yellow rings forming at the top of the tube. The results of each test within the IMViC series were recorded.

<https://assignbuster.com/quantification-of-microbes-in-water-experiment/>

Results

Table 9. 1 - Lactose Testing for Coliforms

Water Sample	DS	SS	SS	MPN	EMB	Lactose
	lacto se broth (10 ml)	lacto se broth (1.0 ml)	lacto se broth (0.1 ml)		resul ts	confirmati on
Littlejohn Lake	5	5	5	> 1600	+	-
Water bottle	2	0	0	4.5	+	-
Lake Byron	5	5	5	> 1600	+	+
Lake Byron	5	5	5	> 1600	+	+
Littlejohn Lake	5	5	5	> 1600	+	-

Littlejohn n Lake	5	5	5	> 1600	+	-
Dog Water	1	0	0	2	+	-

Table 9. 1 illustrates whether the lactose confirmed test for the coliforms were positive or negative as a result of the EMB agar plates examined from the various water samples gathered.

Table 9. 2 - IMViC series results

Water Sample	Indol e	MR -	VP -	Citrat e
Littlejohn Lake	+	-	-	-
Water Bottle	-	-	-	+
Lake Byron	+	-	-	+
Lake Byron	+	-	+	+
Littlejohn Lake	-	+	-	+
Dog Water	-	-	-	+

Table 9. 2 illustrates the results of the IMViC series, indicating a positive or negative reaction for each of the test taken - indole, MR, VP, and citrate.

Discussion/Conclusion

Based on the results shown in Table 9. 1 and 9. 2, my hypothesis - if there is any fecal contamination within our selected water sample, Lake Byron, then the sample will test positive for lactose and differentiate the strains of enteric bacteria showing that it is in fact associated with fecal contamination - is correct. After completing both the lactose and IMViC test, Lake Byron resulted in testing positive for lactose fermentation resulting in the production of both acid and gas meaning that there were harmful pathogens of fecal contamination detected within the Lake Byron water sample. Based on the results in Tables 9. 1 and 9. 2, Lake Byron seemed to be the most fermented water sample meaning it was the most fecally contaminated.

One of the main implications within this experiment would have to be the fact that there were limited resources for each water sample and some IMViC results have some inconsistencies because these series of test may detect genetic strains of a particular species of enteric bacteria coliforms rather than those that aren't as harmful. If I were to replicate this experiment, I would use various water samples from different lakes, water fountains, the Universities' main water stream, etc. to greater evaluate and examine the different coliforms - both harmful and harmless - within the Hattiesburg area as well as on campus. Future experimenters could also provide a wider range of water samples per experimental group to better differentiate the types of

coliforms and provide a more realistically comparable range of water sources to better understand the severity of different coliforms.

References

- Littlejohn, C. (2017). *Microbes in Health & Disease*. Dubuque: Kendall Hunt Publishing Company.
- Martin, N. H., Hsieh, T.-H., Boor, K. J., & Weidmann, M. (2016). The Evolving Role of Coliforms As Indicators of Unhygienic Processing Conditions in Dairy Foods. *Frontiers in Microbiology* , 1-8.
- Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *AMB Express* , 1-16.