Lab 2-Microbial Enumeration

Introduction

There are many different techniques that can be utilized when trying to quantify microorganisms found in a given sample. The purpose of this lab was to gain a better understanding of the advantages and disadvantages of two such techniques, while also presenting students with an opportunity to learn the basic laboratory skills associated with these processes. The first method, a viable count method, allows for an estimation of bacterial organisms that were capable of thriving to grow into visible colony forming units. The second method was a measurement of total DNA concentration. This provides the amount of total detectable DNA present in the given sample. The two different ways of measuring bacterial existence are then able to be compared for reliability and accuracy.

Methods

A single bacteria colony was isolated on Nutrient agar using a streak plating method. From this sample, a pure liquid culture was grown in nutrient broth. This pure liquid culture and a provided environmental sample were then both used to test for total viable counts and total DNA concentration. For both samples a series of dilutions were performed in a 9mL dilution buffer of 0.9% NaCl using 1.0 mL for the pure culture and 0.1 mL for the environmental soil sample in each dilution. A spread plating procedure was performed in triplicate for each sample using the three highest dilutions. Each plate was then counted to estimate the number of CFU/ml of original sample.

 DNA extraction was also performed using 2mL of both the environmental sample and pure liquid culture. Gel electrophoresis was run using both samples and the DNA ladder MBI Fermentas GeneRuler #SM0333. DNA was then quantified with image analysis using the Alphaimager HP as well as by using the Nanodrop spectrophotometer.

Results

Using the plate count data, it was found that there was a much higher number of colony forming units per mL of original sample for the pure liquid culture that there was for the soil sample. In fact, the liquid culture averaged over 43,000 times as many viable colony forming units per mL of sample as the soil sample did.

Plate Count Data

	Liquid	Soil	Liquid / Soil
Average, (cfu/mL)	8.73E+08	2.00E+04	4.37E+04
Std. Dev	1.16E+09	1.51E+04	

The results from the Nanodrop DNA analysis showed very different results from the viable count. When taking into consideration the total amount of DNA in a given sample, there was not nearly as much variation between the pure liquid and the environmental soil samples. The soil sample was actually found to have a slightly higher

average concentration of DNA in ng/µL of original sample than the pure liquid culture. The difference in average concentration was much less significant than it was for the viable plate counts. The concentration of DNA in the liquid culture was about 83% of the concentration of DNA found for the soil sample.

NanodropData

	Liquid	Soil	Liquid / Soil
Average ng/µL	9.2	11.025	0.83446712
Std. Deviation	5.2896	4.3935	

Discussion

      Both of the methods utilized in this laboratory experiment provide us with useful information while still containing some significant drawbacks. The plate count method allows you to estimate the number of the number of cells that were capable of forming colonies on the growth media used, in the time allotted. This is based on the assumption that every viable cell on the plate is capable of growing to yield exactly one colony. Counting the plates based on CFU (colony forming units) expresses results more clearly because a colony-forming unit could easily contain more than one type of cell if the cells were to cluster together. Based on the data collected, this method was much more efficient for the pure liquid culture than the environmental sample because the average number of CFUs on the liquid culture plates was over 10^4 greater than the CFUs counted on the soil sample. The largest bias of this method is the fact that the possible types of bacteria in the original sample most likely have a wide range of conditions that make growth possible. Only the cells which were able to thrive in the conditions we provided with the nutrient agar plates could be counted and included in this data. The use of multiple types of growth media as opposed to only Nutrient Agar, which was our media of choice, can significantly improve the efficiency of plate count methods. This would ensure that many different types of organisms with different physiological needs could be identified, and increase the diversity of bacterial growth.

      The Nanodrop data measured the amount of DNA of any type that could be detected in the sample. This method does not distinguish living cells from dead cells, so it typically results in much higher counts. The amount of DNA found to be present in both the pure liquid and environmental sample was very similar in quantity. These values only differed in a few ng/µL of original sample, nowhere near the 4-fold differences found with the plate count technique. This method is not capable of distinguishing one organism from another, so it does fail to tell us what DNA was detected, whether bacteria or outside organisms not found in the original sample.

      One study performed at Kansas State University emphasizes the fact that testing for total DNA is a much more time efficient method than viable plate counts. This study asserts that the average incubation time needed for growth on a plate is approximately one week. Using DAPI staining produces results much more quickly but the numbers of bacteria present decrease significantly after a few days of storage, varying with temperature and amount of light present during storage.

Conclusion

In summary it was found that the Nutrient Agar plate counting method works best in the following cases:
- When only the quantity living cells are of significance to the experiment
- When using a highly selective growth media for a certain type of organism
- For pure liquid cultures as opposed to the environmental soil samples

A total DNA concentration measurement should be used in the following situations:
- When you need to know the amount of both living and non-living cells
- In laboratory settings but not natural environments
- When you don't need to distinguish between types of specific organisms

Questions
1. *Why is only 0.1 mL plated with the spread plate technique?*
        Only a small amount of solution should be plated with the spread plate technique because a greater volume of liquid might not soak into the plate completely when spread out which would make it impossible to count accurately

2. *Why do we choose to count the series with 30-300 colonies?*
        A plate with less than 30 colonies is not representative of the actual amount of CFUs in the original sample and is not statistically significant. A plate with more than 300 colonies would also probably be too crowded to count.

3. *Explain why the spread plate method yields higher counts from environmental samples than the pour plate method.*
        When performing the pour plate method, you must pipette in some of your environmental sample and then add melted agar on top of it. To be effective, any bacteria in the sample must be able to survive the temperature of the hot agar which is not as likely for the environmental sample used.

4. *Explain why the following statement is true: "The sterile liquid used for making dilutions can simply be water, but a balanced salt solution or growth medium may yield a higher recovery".*
        A balanced salt solution or growth medium may provide a more favorable environment for the growth of many types of bacteria microorganisms. Sodium chloride has been shown to effectively remove some bacteria from the soil during the dilutions, making it more likely to appear in the plating counts.

5. *Using the procedure in this experiment, do you believe you enriched for any obligate autotrophs? Facultative anaerobes?*
        Obligate autotrophs are organisms that require CO_2 as the only source of carbon needed for survival and growth. Because we performed this experiment on plates isolated from large amounts of carbon dioxide, it is unlikely that we enriched for any obligate autotrophs. Facultative anaerobes are organisms that can thrive in environments

lacking any type of oxygen, but can also grow in environments that contain oxygen. Because oxygen is not toxic to these microbes, it is very possible that some of the bacteria we enriched were facultative anaerobes.

6. *Do your plate counts provide a representative estimate of the actual population size and distribution of organisms in your water samples? What is the primary problem with using plate counts for enumeration of microorganisms?*

There are many possible sources of error associated with the plate counts we did for this lab. The plates are an approximation of the amount of organisms in the water samples because we divided our counts by the dilution amount, however the counting is not necessarily accurate all the time. Microorganisms need different types of environments and often different time periods in order to be able to grow into visible colony forming units. Inconsistencies with pipetting resulting from multiple lab members may result in errors or clumps of cell colonies that cannot be reliable counted. The main problem with plate counts for enumeration of microorganisms is the fact that plate counts only represent the number of living colonies and do not consider dead cells.

7. *Compare and contrast the viable plate counting technique with a direct microscopic method, such as DAPI staining. Would DAPI counts be higher or lower than the viable counts obtained from plating? Why? What part of the cell does DAPI stain?*

DAPI counts would be higher than the viable counts obtained from plating because they would measure both living and non-living organisms where plating can only tell us information about living organisms present. DAPI staining stains the nucleic acids present in the cell, thus measuring any type of DNA.

8. *Name on research question or engineering application, each, in which spread plating and DAPI staining would be used to enumerate bacteria. Why would each method be most appropriate for the given example?*

One research application that has shown to be appropriate to use DAPI staining is identifying and quantifying aquatic microflora. Small bacteria and blue-green algae were more successfully identified by using DAPI staining when compared with other methods. It works well because the fluorescent dyes allow particles to be seen that are normally too small to be identified. In engineering, the testing of different water sources for microbes in a laboratory setting would be most effectively performed through DAPI staining because it would give indication to all types of organisms present, not just the ones still living.

Research of any organisms in their natural habitats outside of laboratory would best be quantified by using the plate count technique because staining would identify DNA of outside materials existing in the living environment. An engineering application for use of plate counts would be the analysis of the microbes present in a sewage sample, using a very highly selective growth media. Agar plate counts have also been shown to be an extremely efficient method of detecting Strongyloides stercoralis infection when culturing feces.

References

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