Lab 2: Microbial Enumeration

CE773

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Introduction

There were two acceptable, albeit basic, methods of counting bacteria that were covered in this lab experiment: viable count and total DNA concentration. They are direct and indirect counting methods, respectively. During this lab, the students discovered pros and cons to each of these methods. Upon completion of this lab, one can easily realize why many, many methods exist for counting bacteria, for each method surely has similar characteristics that can excite one person just as quickly as they annoy another (Herrero et al., 2006; Shin et al., 2007).

Methods

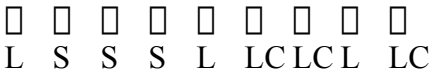
The first step in this lab involved streak plating. Each group was asked to perform streak plating in order to isolate a homogenous source of genetically cloned bacteria. Each group selected 5 colonies that were grown in Lab 1 (Microbial Dispersion in the Environment) and the "T" streak method of streak plating was performed for each colony. Upon completion of streaking, each plate was incubated and stored in the refrigerator. Between classes, a pure liquid culture was grown from one of the streaked colonies.

Next, the total viable count portion of the lab began with the utilization of the spread plate method. Each group chose one pure liquid culture and a soil sample to spread in triplicate. Each sample was diluted by adding 1 mL of sample to 9 mL of dilution buffer. Generally acceptable approximations for dilution levels were chosen based on the environmental origin of each sample. Each of the five highest dilutions was spread plated for each sample, resulting in 15 plates apiece for the liquid culture sample and the soil sample. The plates were then incubated for 24 hours at 30°C. After incubation, 2 mL of each sample were vortexed and centrifuged at 7000 rpm for 5 minutes. Then, the supernant was removed and the bacterial pellet was given to the TA for DNA extraction.

Once DNA extraction had been performed, the DNA was ready to be quantified by gel electrophoresis. The TA prepared the gel for the students and then the students had to load it with the DNA. Three samples of each extract, soil and liquid culture, were placed on the gel, along with three DNA ladders (MBI Fermentas GeneRuler #SM0333), 5 μL each, for positive control. Before being placed on the gel, each sample was mixed with a loading dye to enable the sample to sink through the TAE buffer. A 1 μL sample of extract was mixed with 5 μL of

loader. The ladders (L), soil samples (S) and liquid samples (LC) were placed in the gel slots as illustrated in Figure 1.

Figure 1



Once properly loaded, the gel was hooked up to the power source to run negative to positive. The power source was set to run at 90 mA and 100 V for 60 minutes. (While waiting for electrophoresis, counting of the plates was conducted for the viable count technique.) After the time elapsed, a working stain solution containing 10 μL of 10,000xSYBR Safe Stain and 90 μL of 1xTAE buffer was provided in order to stain the gel. The gel was placed in a stain tray, covered in stain solution, covered with foil, and placed on the shaker table for 30 minutes at slow speed. Finally, the stained gel was ready for Image Analysis. Also, as another method of obtaining DNA concentration data, samples of soil and liquid were analyzed using a Nanodrop Spectrophotometer ND-1000.

Results

Viable Count

Group	Sample	Diluted Plate Count (cfu/mL)	Dilution	Vol. Added (mL)	Original Sample (cfu/mL)
G1	Liquid	85	10^-6	0.1	8.50E+08
	Soil	62	10^-1	0.1	6.20E+03
GC	Liquid	3	10^-6	0.1	3.00E+07
	Soil	300	10^-1	0.1	3.00E+04
MNG	Liquid	9	10^-6	0.1	9.00E+07
	Soil	80	10^-1	0.1	8.00E+03
JJJ	Liquid	126	10^-6	0.05	2.52E+09
	Soil	179	10^-1	0.05	3.58E+04

The above table shows the average liquid and soil plate count data from each of the four groups. Using the dilution and volume added, the number of colony forming units (cfu) per 1 mL of original sample was calculated. When taking all groups into account, the average number of cfu/mL for the original liquid sample was 8.7E+08. Likewise, the average for the soil sample was 2.0E+04 cfu/mL.

Total DNA Concentration

Image Analysis

Ladder Area	
(measured by peak intensity)	
Ladder 1	185183
Ladder 2	72856
Ladder 3	110001
Average Ladder Area:	122680
ng DNA/area:	0.00049

Sample 1 - Soil			
(area measured)		(area measured/5 µL)	(ng/µL DNA extract)
Area 1:	69792	13958.4	6.83

Only one group (JJJ) obtained a successful image analysis showing DNA. From the image, an average ladder area of 122680 was calculated. Based upon the known value of 60 ng DNA per 0.5µg ladder, a value of 0.00049 ng DNA/area was then calculated. Finally, using this calculated value, a concentration of 6.83 ng/µL DNA extract was calculated for the soil sample.

Nanodrop

Group	DNA Conc. (ng/µL)	
	soil	liquid
G1	10.3	9.7
GC	5.2	6.4
MNG	15.3	4.3
JJJ	13.3	16.4

The average soil DNA concentration for all four groups was 11.0 ng/μL. For liquid, the average concentration was 9.2 ng/μL.

Discussion

The results of this experiment bring about a notable difference between the viable count method and the DNA concentration method. In the viable count, the average soil concentration was 2.0E+04 cfu/mL, compared to 8.7E+08 cfu/mL. So, the soil concentration is only 0.002% of the liquid concentration. These results give credit to doubts that have been raised about traditional agar plating's ability to successfully mimic nature (Johnson et al., 1999) when compared to the DNA concentration results. When looking at the Nanodrop DNA concentration data, one can see that the average soil DNA concentration is 120% of the average liquid concentration. Therefore, the viable count method would have one believing that soil concentration is much lower than liquid concentration, while the DNA concentration method would lead to the opposite observation.

The main reason behind these widely varying results between methods could be the agar used in the viable count. It has been shown in the past that different agars can produce different concentrations of bacterial growth (Lowe et al., 2006). So, the single agar selected for this experiment probably limited the amount of bacteria that was able to grow successfully. Whereas, the DNA concentration method does not have such a limiting factor present in its process.

Overall, the results of this lab experiment help create an understanding of the variables present in bacterial counting methods. The viable count method appears to be vulnerable to greater sources of error, such as pipetting, proper mixing, etc., which could skew its results.

Conclusion

The basic reality demonstrated in this lab was that no two bacterial counting methods will produce similar results, and the differences between any two methods could be rather significant. Upon analyzing the results, one could say that the DNA concentration method may be the most reliable method across all types of samples. Also, if the viable count method must be used, one

should at least consider using several agar types in order to yield the best results. Utilizing multiple counting techniques when trying to quantify bacteria concentrations, as is often done in research, is likely the best approach (Pujol, 2006).

References

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Questions

1. Why is only 0.1 mL plated with the spread plate technique?

   With greater sample volumes, the excess amount of liquid does not soak in. This may cause the colonies to coalesce as they form and make them difficult to count.

2. Why do we choose to count the series with 30-300 colonies?

   If the number of colonies is too large, some crowded cells may not form colonies and some colonies may fuse together, leading to erroneous measurements. Also, if the number of colonies is too small, the statistical significance of the calculated count will be low.

3. Explain why the spread plate method yields higher counts from environmental samples than the pour plate method.

   Not all the organisms present in the environmental sample may be able to withstand the temperature of the molten agar used in the pour plate method. Therefore, fewer counts would result from the pour plat method.

4. Explain why the following statement, found in Figure 6.11 legend (p.146) of your text 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".

   The added nutrients present in the salt solution or growth medium dilution could help keep more of the bacteria viable during the dilution process. This would result in a higher yield.

5. Using the procedure in this experiment, do you believe you enriched for any obligate autotrophs? Facultative anaerobes? Discuss.

   Any organism that had aerobic abilities, be they obligate or facultative, probably had an advantage in this experiment since oxygen was present in the procedure. I do not believe that obligate autotrophs or facultative anaerobes had a similar advantage and, most likely, they had a marked disadvantage to aerobes.

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?

   No. Plate counts can reproduce a certain subset of organisms present in an environmental sample, but, due to the specific nutrient medium and growth conditions used, the total cell count is generally a gross underestimate of the environmental population as a whole.

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?

   The DAPI method would be higher because the DAPI method would count viable cells *and* dead cells. Also, DAPI staining is not subject to the nutrient and envirionmental requirements of the viable plate counting technique, so all types of organisms could be counted. The DAPI staining method stains the DNA.

8. Name one 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?

   Spread plating would work well in the food industry when testing a sample for the concentration of a specific microbiological toxin. The culture media and growth medium could be tailored to fit the needs of the desired bacteria.

   DAPI sampling would work well for water stream sampling, when the types of bacteria present could vary greatly and a relatively accurate count of all microorganisms present is desired.