

Lab 2 - Microbial Enumeration

I. Introduction

Viable counts and total counts are two basic methods to enumerate bacteria. A lot of studies have been done showed the different advantage and disadvantage of them^[1]. This lab used these two counting methods: viable counts by spread plating and total counts by DNA gel electrophoresis, to show the difference of microbial concentration in pure liquid culture and in soil solution. And there were two kinds of water sample used in the experiment, environmental sample--soil solution and pure liquid culture sample. Some former study found that bacteria in soil solution has a higher total counts than in pure culture^[2]. However, an opposite result was found in this experiment that pure culture has higher total and viable counts than soil solution. On the other hand, the difference between the two counting methods was also showed in this lab. Viable counts had a much more significant difference between these two samples than total counts.

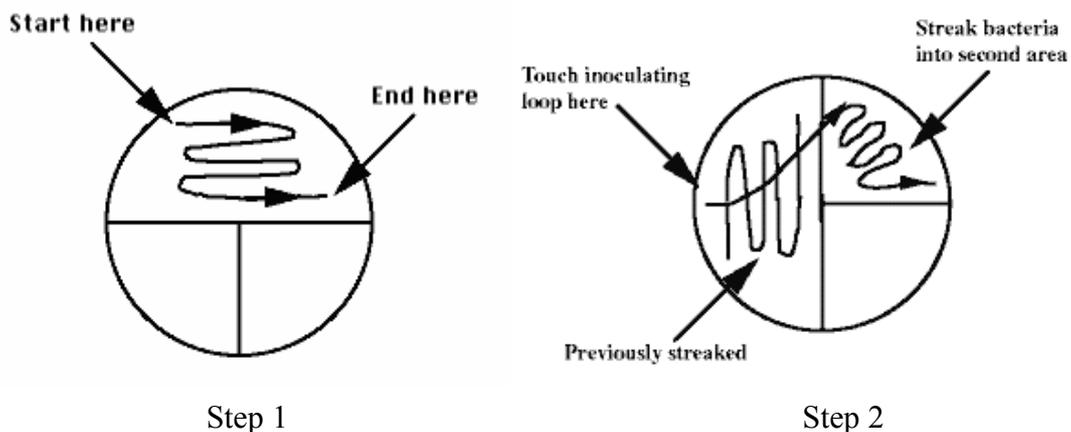
Another new device was also used for total counting: "Nanodrop", which can measure the concentration of DNA and RNA by measuring the absorbance of DNA, RNA with extremely small sample volume^[3].

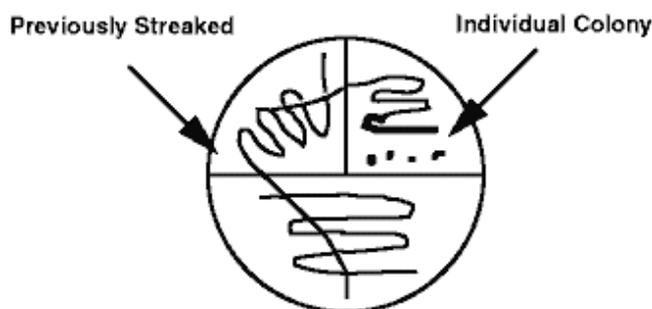
II. Method

Streak Plating

Firstly, we poured the hot solution provided by TA, which contains nutrient agar, into 60 plates quickly, and waited about 20 minutes to let the solution in plates harden. Then we put all the plates into plastic bags up side down, and gave them to TA, who will store them in the refrigerator for further use.

Secondly, we used nutrient agar plates provided by TA, which were made by the same method that we did, to do the streak plating. We lighted a Bunsen burner before the operation to make sure the whole streak plating is in sterile conditions. The streak plating method we used is shown as in Graph 1, which is called the "T" streak.





Step 3

Graph 1 – “T” streak procedure

Before each Step (1, 2 and 3), we held the inoculating loop’s tip in the burner’s flame to sterilize it, and after heating, we poke the tip into clean agar to cool it. In Step 1, we touched one single colony on a cultured plate we made before with the loop’s tip. Then, we put the tip on the agar surface of a new plate, and moved softly as the way we showed in Graph 1. In Step 2 and 3, we moved the loop tip on the agar surface of the same plate as the way in Graph 1 after the sterilizing and cooling. After Step 3, we heated the loop tip again to sterilizing it.

We did streak plating 5 times, and gave that 5 plates to TA, who will incubate it in the incubator for about 1~2 days and then store it in the refrigerator for further use.

Enumerating Viable Cells

We used two kinds of sample: soil sample and liquid culture sample to operate ratio dilution by dilution buffer, which is clean water with balanced salt to. The liquid culture we used was grown from one of our streak plates.

For the dilution, we pipetted 1 mL of the original sample into 9 mL dilution buffer, and vertexed the new solution, which generally made a 10 times dilution. Then, we pipetted 1 mL of the new solution into another 9 mL dilution buffer, and vertex, which totally made a 100 times dilution from the original one. And so forth, till we got the concentration we need.

After the dilution, we used the three highest dilution rate solutions to do the spread plating. For both kinds of sample, we used the solutions with dilution rate of $10^{-5}/\text{mL}$, $10^{-6}/\text{mL}$ and $10^{-7}/\text{mL}$. However, the soil samples turned out to be over diluted in the next lab, which had nothing grown on the spread plates. Since that, we redid the spread plating with original concentration of the soil sample, which had a dilution rate of $10^{-1}/\text{mL}$.

For the spread plating, we lighted a Bunsen burner before the operation to maintain a sterile condition. We pipetted 0.1mL diluted solution on the surface of an agar plate, which was put on a turntable. Then we used a bent glass hockey stick, which was sterilized (the stick was put in alcohol, and then placed in flame to let the alcohol burn off) and cooled to spread the plate by putting it on the surface of the plate and moving back and forth with turning the turntable. After the spread plate, we sterilized the

hockey stick again.

We did 3 times of spread plating for each of the dilution rate and each kind of sample. Then we waited to let the solution soak into nutrient agar, and gave them to TA, who will incubate and store them for further use.

After about 2~4 days, we counted the number of colonies on the incubated spread plates we made and reported the number and dilution rate of each sample.

DNA Concentration Test

We pipetted 2mL of each kind of sample (soil and liquid culture) into a 2mL sterile microcentrifuge tube separately. Then we vortexed the tubes and centrifuged them by a microcentrifuge at 7000 rpm for 5 minutes.

After centrifuge, we poured off the liquid in the tubes carefully and gave the tubes with bacterial pellet to TA, who will use them to do the DNA extraction for the DNA gel electrophoresis.

For the DNA gel electrophoresis, the gel had been made and put into the gel electrophoresis apparatus with the correct position by TA. We firstly poured TAE buffer into apparatus till the whole gel was in the buffer. Then we put a piece of parafilm on the bench with sterile side up, and pipetted samples, ladders and loading dye solutions on that. For ladders, we pipetted 5 μ L and for samples, we pipetted 5 μ L of sample and 1 μ L of loading dye solution, and wait to let them mix completely. Then, we pipetted each of these solutions into a well on the gel. We totally made 3 ladders and 3 of each kind of sample.

After that, we put the lid on and connected the apparatus with the controller by wires. We ran it with parameters of: 60min, 90mA and 100V.

One hour later, we took out the gel and put it into a container, and poured SYBR safe stain solution into the container till the whole gel was in the solution. Then we put the container on a shaker table and covered it with aluminum foil to avoid light.

We shake it for about 30 minutes, and then brought the gel to the gel perform image analysis.

However, the image we got showed that there is no significant band of our samples, which made the image analysis impossible. Since that, we used another DNA analysis device named “Nanodrop” to measure the total counts.

III. Results

Table 1 - Viable Counts Based on Enumerating Viable Cells

Sample	Bacteria Concentration (cfu/mL)
Liquid Culture	8.73E+08 \pm 1.16E+09
Soil	2.00E+04 \pm 1.51E+04

Table 2 - Total Counts Based on Nanodrop Test

Sample	DNA Concentration (ng/ μ L)
Liquid Culture	11.03 \pm 4.39
Soil	9.20 \pm 5.29

The data I got shows a very high standard deviation in both viable counts and total counts, especially the former, which highly declines the confidence level and accuracy of our result. This is mainly attributed to the differences among the groups' operation.

However, I still can find from the data that both viable counts and total counts of liquid culture sample have a higher average than soil sample's, based on which I assume that liquid culture has a higher microbial concentration than soil.

On the other hand, the difference of viable counts between liquid culture and soil is much more significant than total counts.

IV. Discussion

The high standard deviation in the result reveals our lack of lab operation skill. Since one of the main purpose of this lab is to make students familiar with microbiology lab operation, this result is still acceptable, because that I did learn useful things from this lab and improved my lab skill.

There are two valuable results I got from the data.

Firstly, the biomass of pure liquid culture is higher than soil solution. Since the bacteria in the pure liquid culture is grown from the bioreactor plate, not the soil plate. I am not sure that there is the same kind of bacteria exists in the soil. I assume that it does exist in the soil. Thus from the result, I find that this species has a higher biomass in pure liquid culture than in the soil, which can probably be explained by factors of competition or predation between species in mixed culture (soil solution). Possibly, there are some other kinds of organisms in the soil solution closely related to the certain kind of bacteria we tested and with similar modes of existence, which will make the bacteria hardly to inhabit because of competition factors^[4]. Or possibly, there are some other kinds of organisms in the soil solution can predate on other soil microorganisms under nutritional limitation^[5], which will also decrease the biomass of the bacteria we tested. Another simple reason for difference of microbial concentration in these two kinds of sample is that the growing conditions of the two samples like temperature, pH and pO_2 are different, and the liquid culture condition is better for that kind of bacteria.

Secondly, the difference of viable counts between liquid culture and soil is much more significant than total counts. There are three possible explanations for this difference. One reason is that because of the inappropriate operation of enumerating viable cells, the data I got for the viable counts is far away from the true value. Another reason is that Nanodrop has some unknown weakness in measuring total counts, like it cannot measure pure culture or mixed culture accurately. Despite it seems to be an advanced technology for DNA and RNA test, it is a new method, and the unknown disadvantage of it should be considered. Finally, Nanodrop measured both living and dead cell's DNA, but for viable counts, it just measures the amount of living bacteria. Since that, it is possible that there is a much larger amount of dead cells in the soil solution than the pure liquid culture, which is just incubated several days.

V. Conclusion

From this lab, I find that:

- Pure liquid culture has a higher microbial concentration than soil solution, which supports the theory that pure culture is better than mixed culture for bacteria to grow.
- Viable counts and total counts do not have a direct ratio, which probably is caused by the different ratio of living cells to total cells in the two kinds of sample.
- The new device “Nanodrop” used in this lab has an impressive efficiency in DNA concentration measuring.

This study does have the value to go further, just need to be more accuracy.

VI. Reference

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VII. Questions

1. Because that the solution is highly concentrated with bacteria, although it is diluted. Thus, if we use too more solution on the spread plate, there will be too many bacteria colonies on the plate, which makes it impossible to count the right number.
2. Because low number of colonies will increase coincidence of data, which leads to higher error. And high number of colonies is hardly to count, which also leads to higher error. In sum, we choose to count the series with 30~300 colonies to minimize data error.
3. In this lab, spread plate method yields higher counts from pour plate method than environmental samples. There are two possible reasons for this result. Firstly, the

factor of competition and predation between species in mixed culture of environmental samples restricts bacteria to grow. Thus pour plate method, which is a pure culture method, will field more counts. Secondly, the living conditions (like temperature, pH and pO_2) of pure culture maybe are better than environmental mixed culture.

4. The buffer solution with balanced salt is better than water because that the buffer solution have a much more appropriate concentration of salt than water for bacteria to live. Extreme low salt concentration water will overfill into bacteria's cell, which is harmful to bacteria, even kill them.
5. Since we used nutrient agar as the culture medium, which contain a lot of nutrient material, I did not enriched for obligate autotrophs. And for facultative anaerobes, since it can grow no matter with or without oxygen, I may enrich for some of them, but not intentionally.
6. I think our plate counts are generally representative for the actual population size of water samples, but not distribution of organisms. The primary problem with using plate counts for enumeration of microorganisms is that it can just give a generally estimate of the population size of samples, but cannot provide the distribution of organisms of a mixed culture.
7. Viable plate counting technique can just generally count the number of living bacteria. However, DAPI_[6] can stain both live and fixed cells, which makes this technique can count the number of live and fixed cells. Since that, DAPI counts probably will be higher than the viable plate counts. DAPI stains cell's DNA.
8. Research question: Determine the productivity of a kind of bacteria in certain condition.

Engineering application: Measure the microbial concentration in the water sample. In the first case, DAPI is the most appropriate method to enumerate bacteria, because that this method can count both live and fixed cells, which is exactly the total productivity of the bacteria.

In the second case, spread plating is the most appropriate method to enumerate bacteria, because that microbial concentration only need count the living bacteria, which we can get from spread plating.