CE 773 Lab 1

Microbial Dispersion in the Environment

Introduction

In order to examine microorganisms thoroughly without the use of a microscope, they must be given the proper conditions and nutrients and allowed to flourish into colonies. In this experiment, microorganisms were taken and cultivated from nine different sources. For each of these nine sources, there were three different media types used – nutrient agar, luria broth agar, and luria broth with tetracycline added. The intent was to determine the differences between the different media types used and the different colony counts observed, while at the same time appreciating the need for sterile techniques to combat contamination.

Methods

The methods used include, first, preparing the agar plates. There were four groups with three members taking place in the experiment and each group used 21 plates; 9 of luria broth agar, 9 of luria broth plus the inhibitor tetracycline, and 9 of nutrient agar. To prepare the plates, the appropriate mass of media (luria broth or nutrient agar) was melted in 500 mL of distilled water using a hot plate and stir bar. The solutions were then autoclaved for 15 minutes at 121°C. When the media had cooled enough to easily handle, 16 mg/L of tetracycline was added to one of the two flasks of luria broth. The plates were then poured and allowed to cool.

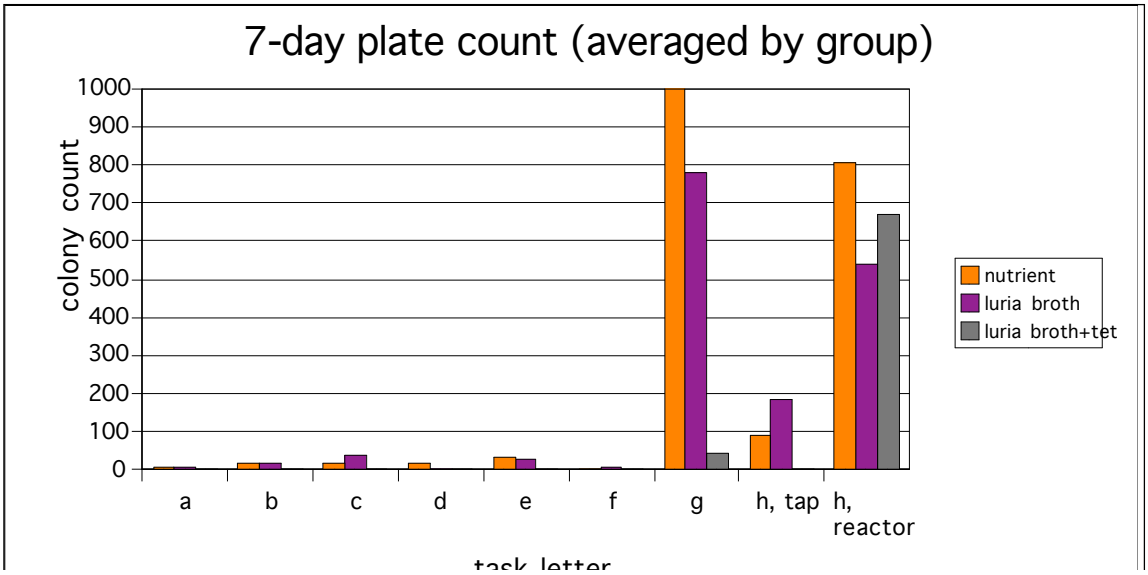
After cooling, the experiment in the prevalence and growth patterns of microorganisms could begin. One person from each group performed 9 tasks (associated with letters a through h) using 9 plates of either nutrient agar, luria broth agar, or luria broth plus tetracycline. The first and second tasks were to uncover the plates and allow to sit exposed to air for 15 and 45 minutes, respectively. The third plate was streaked with a cotton swab which was wiped on the surface of the laboratory

bench. Next, respectively for the fourth and fifth plates, the plates were uncovered and then each person shook their head vigorously over the plate and touched the surface of the plate. The sixth plate was coughed or sneezed on. The seventh plate had a soil slurry mixture (1 gram of soil and 10 mL sterile distilled water) spread onto it. The eighth and ninth plates had two different samples of water (1 mL tap and bioreactor water into 10 mL sterile dilution water) spread over it. Seven days after the plates had incubated at room temperature in a dark cupboard, the plates were examined and the colony counts tabulated.

Sterile techniques were used throughout the lab. For a more detailed listing of the procedures, see handout associated with the lab.

Results

	nutrient	luria broth	luria broth+te
a	8	7.25	0.5
b	17.75	18	1
c	16.25	36	0.25
d	16.75	3.5	0.25
e	31.33	29.33	1.67
f	1.25	5.5	0
g	998	778.67	43
h, tap	92.5	182.75	4.25
h, reactor	807	538.5	670



Along with the observable bacterial colonies that grew on the plates, there were fungus colonies, which looked like fuzzy bacterial colonies. The fungus counts did not follow a specific pattern. All three media used grew comparative amounts of fungus, mostly on task 3. As for the bacterial colonies, they had the highest count in the soil and bioreactor water. As can be seen in the chart, the tetracycline was effective at inhibiting much of the bacterial growth. The organisms from task b cultivated red bacteria and organisms from task c cultivated yellow bacteria. Streaking of the plates, which was done on the last three tasks, grew bacteria that was very hard to count because of its prevalence on the plate.

Questions

1. Why is it necessary to sterilize the liquid media in a humid, pressurized environment (as opposed to a hot oven)? Would it be possible to sterilize dry glassware in an unpressurized oven? Does the autoclave truly "sterilize" the media?

   Pressure caused from the water vapor inside an autoclave directly corresponds to the temperature in the contained area. The higher the pressure, the higher the temperature; and the more saturated vapor that is inside the autoclave, the less time and lower the temperature has to be. If the liquid media was put in a hot oven to be sterilized, it would reach boiling point, in which case the water would begin to evaporate thus ruining the media. A few strains of bacteria would be killed by heating to just under boiling point in a hot oven, but this proves to be a much

less effective sterilization technique. As far as dry solid media, such as glass, goes, it is possible to sterilize it in an unpressurized oven, but a higher temperature must be reached and it must be in the oven for a longer amount of time. Dry heat does not have the ability to works its way into the media as efficiently as does wet heat.

2. Is either of the water sources tested "sterile"?

1 mL of tap water and bioreactor water were added to 10 mL "sterile" water (separately) and then smeared on the plates (represented under h,tap and h,reactor on the graph). To be sterile would require the water to be free of most, if not all, microorganisms (specifically bacteria and viruses). As can be shown on the above graph, the colony counts for the tap water and bioreactor water were among the highest. As such, it can be concluded that even though sterile methods were used for transferring the dilution water and for containing the water sources, the water smeared on the plates and evaluated after seven days incubation were not sterile.

3. Why must "sterile" techniques be employed when performing pure culture experiments in a lab like ours? What would have happened if we didn't use "sterile techniques"? Under which conditions are "sterile techniques" more critical – when you are working with autotrophic or heterotrophic pure cultures?

As our growth cultures can attest, there are microorganisms everywhere. Sterile techniques allow us to be more accurate in the identifying of microorganism species. If sterile techniques hadn't been used, the resulting colonies forming on the agar would have potentially come from any number of sources. In actuality, it is very hard to ensure that the colonies grown on the plate are from the original source, even with or without using sterile techniques.

Autotrophic organisms are considered 'primary producers', meaning they use CO_2 as their sole source of carbon. Heterotrophs, on the other hand, live off the primary producers or the products excreted by primary producers. This being said, it seems more important to make sure sterile techniques are used with autotrophs because they do not use other organisms for growth. Heterotrophs would potentially be able to use any microorganisms they come in contact with during the cultivation process.

4. Explain the principle behind the use of the Pasteur flask in studies on spontaneous generation. Explain why the invention of a solid culture media was of great importance to the development of microbiology as a science.

In Pasteur's time, it was claimed that microorganisms spontaneously appeared on food. He disproved this theory by inventing the Pasteur flask. The idea being that a nutrient solution placed in the flask, then heated to boiling thus forcing the stale air out would prevent the solution from putrefying. The flask had a bent neck to trap microorganisms and prevent them from entering.

The invention of solid media, specifically agar, was enormously effective at obtaining more well-defined cultures. Before agar the best culture medium was gelatin, which did not stay solid at 37 degrees Celsius – the "optimum temperature for growth of most human pathogens." Also, most microorganisms cannot degrade agar. Agar opened a whole new, more effective way to observe and study the patterns of growth in microorganisms.

5. In theory, what does a single, isolated colony on an agar plate represent?

A single, isolated colony on an agar plate represents a pure culture – meaning growth of only a single kind of microorganism.

6. Would a bacterial colony continue to increase in diameter (indefinitely) upon incubation?

Explain why or why not.

No - eventually the bacteria would run out of nutrients with which to metabolize and become static.

7. Discuss the differences of luria broth agar and nutrient agar. What purpose does the agar serve? Did the media have any effect on microbial growth? Discuss why different media are commercially available?

   The agar serves as a semi-solid medium which provides the perfect setting for quick bacteria growth. Different types of media are more apt to grow and isolate different types of bacteria. There are many different kinds of media available, most harboring certain nutrients known to encourage growth of certain microorganisms. Two broad categories are chemically defined media wherein the exact chemical composition is known and complex media made up of highly nutritious but chemically undefined substances. Selective media, like the luria broth with the tetracycline inhibitor, is a complex media used to inhibit growth of some bacteria while supporting growth of others. Also, there is differential media wherein a dye is added to the mixture to distinguish certain chemical reactions occurring during growth.

8. Describe an enrichment media and physiological conditions that could be used to isolate the following:

   i). Staphylococcus aureus: This organism is best grown on nutrient agar or nutrient broth at 37°C. Any media that inhibits growth of gram-negative bacteria but encourages the growth of gram-positive bacteria would be suitable.

   ii). Thiobacillus ferroxidans: An agar with high ferrous iron concentrations would be best for this organism. The optimum pH for cultivation lies between 2.5-5.8 and the optimum temperature is around 15-20°C.

iii). Mycoplasma mycoides: As a facultative aerobe, this organism will grow best in aerobic conditions at 37°C. The pH should be around 6.0-7.8. According to Biology of Microorganisms, most mycoplasmas use carbohydrates as carbon and require a range or vitamins, amino acids, purines, and pyrimidines for growth. Thus, a chemically defined media would be best.

9. Discuss the influence of the inhibitor on the observed plate counts and dominant organisms from the different sources. Refer to your tabulated data from the whole classes' samples. What is the mechanism of inhibition for the selective inhibitor used in this lab? What type of organism does this inhibitor suppress?

   The tetracycline used in the agar plates acts as a protein synthesis inhibitor, "interfering with 30S ribosomal subunit function." It suppresses the protein synthesis of gram-negative, gram-positive, and obligately parasitic Bacteria. It's hard to see in the above graph, but the tetracycline inhibitor worked remarkably well in reducing the colony counts of all plates except the plate smeared with water from a bioreactor.

Conclusion

The results show that the differences in the media used to grow the various organisms had a significant impact on the number of colonies at the end of the incubation period. Except for the bioreactor water, the organisms grown on the luria broth plus the added tetracycline inhibitor were considerably fewer. It can be assumed that the bioreactor water contained a microorganism which was out of scope of the tetracycline's protein synthesis suppressing powers. The luria broth and the nutrient agar were comparable in their ability to provide good growth conditions for various organisms. Streaking of the plates which was done with tasks g, h tap, and h reactor proved to be less effective

when trying to isolate single colonies. Furthermore, using sterile techniques allowed us the ability to examine these microbe colonies with minimal interference from outside organisms.

References

1. Buchanan, R. E., and N. E. Gibbons. Bergey's Manual of Determinative Bacteriology. 8th ed. Baltimore: Williams & Wilkins Co., 1975.

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3. Madigan, Michael T., and John M. Martinko. Brock Biology of Microorganisms. 11thth ed. Upper Saddle River, NJ: Pearson Prentice Hall, 2006.