Lab Report 1

Microbial Dispersion in the Environment

INTRODUCTION:

The natural environment is populated with many microorganisms suspended in the air, in water, and associated with other organisms (e.g., humans). This is of particular importance to laboratory work in microbiology. All surfaces in the lab and in the environment are potential sources of contamination in microbial experiments. Because of the many sources of contamination, special methods, called "sterile techniques", have been developed to minimize laboratory contamination. One learning objective of this first lab is to gain an appreciation for the abundance of microorganisms in the environment and the ease of microbial dispersion.

Microorganisms from any source can easily be grown and visualized in liquid or solid growth media. The purpose of any microbial growth medium/environment is to provide the nutritional (carbon source, nitrogen source, etc.) and physicochemical conditions (pH, redox levels, temperature, etc.) required to maintain the viability of the population. Each type of microorganism, however, has its own preferred nutritional, physicochemical conditions, and antimicrobial susceptibilities; therefore, the variety of media used in microbiology can be very broad. This experiment assesses the effects of sample source and growth media on the number of microorganisms that the can be enriched from similar sources.

METHODS:

Pouring Plates

TA melted the appropriate mass of media in 500 ml of distilled water (placed in an Erlenmeyer flask covered with aluminum foil) using a hot plate and stir bar, and prepared this for each of the following: (1) 2 x Luria broth agar; (2) 1 x Tripticase soy agar. The hot solutions were transferred to the autoclave, and sterilized for 15 minutes at 121^{o}C and 18 psi. The hot media was taken out of the autoclave (keep covered) and placed on a stir plate with slow stirring. During cooling, the bottom of the Petri dishes were labeled as follows:

10 plates “LB” ……..…..LB = luria broth
10 plates “LB Tet”..…….... Tet = tetracycline
10 plates “NA”…….... NA = nutrient agar

Once cooled, 16 mg/L of tetracycline was added to the LB in which “Tet” was labeled. Then TA poured ten plates for each solution, it took fifteen to twenty minutes to let the agar harden, and then stored the solidified plates, inverted in plastic bags, in the refrigerator.

Transferring Environmental Organisms

Each of us was given a set of plates as described above. The plates have been removed from the refrigerator and are sitting on the bench to warm up, permitting condensation to evaporate. Then we performed the following tasks and labeled each plate: a. allow the plate to sit exposed to the air for 15 minutes; b. leave another plate exposed to the air for 45 minutes; c. streak the entire surface of the third plate with a zig zag motion with the cotton swab which wiped the laboratory bench; shake head vigorously over the fourth plate surface; e. touch the surface of the fifth plate with fingertips in several places; f. cough or sneeze onto the sixth plate surface; g. suspend 1 gram of soil into 10 ml of sterile distilled water and vortex the solution for one minute, then sterilely transfer 50 µl onto the seventh plate and sterilely spread the material equally across the plates with a spreader; h. sterilely transfer 1 ml of tap water sample and bioreactor water sample into 10 ml of the sterile dilution water, then spread 50 µl of each water sample onto separate plates (plates 9 and 10). These plates were then incubated in the dark in the cupboard below the lab bench for seven days.

Observations

We examined the plates and tabulated each plate counts. The size, color, shape, and texture of the colonies were observed from each plate. Plate counts from the other groups were gathered too.

RESULTS:

The following table shows the plate counts from all the groups. The numbers show the amount of bacteria, the numbers with ( ) show the amount of fungi.

Treatment	Group	NA	LB	Tet
a	A	8	5	2
	B	8, (1)	8, (8)	(2)
	C	11	8	0
	D	5	8	(2)
b	A	12	21	3
	B	24, (3)	9, (8)	0
	C	12, (3)	33	1, (4)
	D	23, (2)	9	(4)
c	A	10	3	1
	B	53, (3)	112, (25)	(3)
	C	2	13, (2)	(2)
	D	0	16	(1)

d	A	0	3	1
	B	11, (1)	2, (1)	0
	C	0	7	0
	D	56	2	0
e	A	25	62	3
	B	60, (1)	19, (9)	2
	C	9, (4)	7	0
	D	Broke	28, (1)	(2)
f	A	0	1	0
	B	0	(2)	0
	C	3	18	0
	D	2	3	(2)
g	A	1152	Missing	31
	B	840	560	46, (6)
	C	800	800	52, (7)
	D	1200	976	21, (6)
h, tap	A	85	97	5
	B	123	144	5
	C	50	400	6
	D	112	90	6
h, reactor	A	948	24	576
	B	960	616	804
	C	600	650	800
	D	720	864	560

Based on my plate, there are two distinct colonies developed on plates, they have different size, color shape and texture: The bacteria is mostly granular and has the diameter less than 1 mm. It is much smaller than fungi, whose diameter could be 8 mm. The bacteria have smooth edges, while fungi have hairy ones. The fungi also have round shape, but it is more colorful than the bacteria. The bacteria on my plate have only one color, which is near the color of agar; some fungi are yellow, some are dark green.

CONCLUSION

Microorganisms are everywhere – in the water, in the air, in the animal body, and so on. They can easily spread to the environment in which they prefer. There are different types of microorganisms growing on luria broth agar and nutrition agar under the same physicochemical conditions, for those two kinds of agar provided different nutrition. Tetracycline could suppress the growth of bacteria, but not do much to the fungi.

QUESTIONS:

1. Why is it necessary to sterilize 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?

S: An autoclave is a large pressure cooker; it operates by using steam under pressure as the sterilizing agent. High pressures enable steam to reach high temperatures, thus increasing its heat content and killing power. Most of the heating power of steam comes from its latent heat of vaporization. The ability of air to carry heat is directly related to the amount of moisture present in the air. The more moisture present, the more heat can be carried, so steam is one of the most effective carriers of heat. It is not possible to sterilize dry glassware in an unpressurized oven. Moist heat is thought to kill microorganisms by causing coagulation of essential proteins, so the autoclave truly sterilizes the media.

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

S: The water sample from the tap and bioreactor are neither sterile. There are lots of bacteria and fungi living in water. People can drink tap water not because it is sterile, but the bacteria and fungi in it won't cause human's sickness, some of those are even good for people's health. The bioreactor uses bacteria to clean water; some of them may still be in the discharged water.

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? Discuss.

S: Because bacteria and fungi are all around us, in the air, in the water, on our finger, everywhere. If we didn't use sterile techniques, some unexpected bacteria and fungi will grow in our plates, which could cause a lot of trouble to distinguish, and they may affect the growth of the aimed bacteria. I think sterile techniques are more critical when working with heterotrophic. Because if there are other bacteria growing in the plates, competing food won't affect autotrophic bacteria a lot, but it may cause heterotrophic cultures to die because of hunger.

4. (Connect to textbook, p.20) 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.

S: Pasteur denied spontaneous generation by demonstrating that life does not currently spontaneously arise from nonlife in nature. It is because bacteria can't live when heated to boiling behind this experiment – protein is damaged under high temperature so that bacteria can't live.
Solid medium is media containing agar or some other, mostly inert solidifying agent. It has physical structure (broth lacks structure) and this allows bacteria to grow in physically informative or useful ways. Because bacteria could meet nutrient, air and light which they need easier; and it is more convenient for observation and research.

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

S: It means there is a pure culture in the plate.

6. Would a bacterial colony continue to increase in diameter (indefinitely) upon incubation? Explain why or why not.

S: No, because when new bacteria grow, old ones die. When it gets to the balanced point, the colony will stop increase in diameter.

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.

S: The nutrient elements in these two agars are different; it can result in different microorganism growth, even from the same source. Agar is used as a solid culture media. The media does affect the microbial growth. The microorganism depends on the nutrition in the media, different media grow different microorganisms. If we know the aim bacteria like one kind of nutrition, we could choose a media which is rich in the special nutrition and it will help the bacteria grow, so different media are commercially available.

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

(1) Staphylococcus aureus

S: Nutrient agar or nutrient broth

(2) Thiobacillus ferrooxidans

S: Acidithiobacillus ferrooxidans medium:

Solution A: ml

(NH4)2SO4.....0.8 g

MgSO4 . 7H2O .....2.0 g

K2HPO4 .....0.4 g

Wolfe's Mineral Solution (see below).....5.0

Distilled water.....800.0 ml

Adjust Solution A to pH 2.3 with H2SO4.

Filter-sterilize.

Solution B:

FeSO4 . 7H2O .....20.0 g
Distilled water.....200.0 ml
Stir Solution B to dissolve and quickly filter-sterilize.
Aseptically combine Solutions A and B. (A yellow precipitate is normal; it becomes darker as the iron oxidizes.)
Wolfe's Mineral Solution:
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS).
Nitrilotriacetic acid.....1.5 g
MgSO4 . 7H2O .....3.0 g
MnSO4 . H2O .....0.5 g
NaCl.....1.0 g
FeSO4 . 7H2O .....0.1 g
CoCl2 . 6H2O .....0.1 g
CaCl2 .....0.1 g
ZnSO4 . 7H2O .....0.1 g
CuSO4 . 5H2O .....0.01 g
AlK(SO4)2 . 12H2O.....0.01 g
H3BO3 .....0.01 g
Na2MoO4 . 2H2O.....0.01 g
Distilled water.....1.0 L
Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

(3) Mycoplasma mycoides

S: Mycoplasma medium: For broth, omit agar from formula below.
Heart Infusion Broth (BD 238400).....17.5 g
Noble Agar (BD 214230) (if desired) .....10.0 g
Distilled water ..... 700.0 ml
Autoclave at 121°C for 15 minutes. Cool to 55°C (or room temperature for liquidmedium) and aseptically add the following filter-sterilized solutions:
Horse serum (heat-inactivated) ..... 200.0 ml
Yeast Extract Solution (GIBCO 18180).... 100.0 ml

9. Discuss the influence of the inhibitor on the observed plate counts and dominant organisms (e.g., bacteria, fungus etc.) 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?

S: From the data, the inhibitor used in this experiment suppress the grow of bacteria, it didn't do much on fungi. Tetracyclines bind reversibly to the small subunits of bacterial (and eukaryotic) ribosomes where they interfere with binding of charged-tRNA to the "Acceptor" site. They are "bacteriostatic" rather than cidal. It suppress the bacteria rather than fungi.