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). All surfaces in the lab and in the environment are potential sources of contamination. The ease in which microorganisms may be dispersed in the environment has significant consequences on the quality of laboratory measurements that may wish to examine a particular cell.

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:

Four groups with three members performed identical plating of all samples on three different types of agar plates: luria broth agar, luria broth agar with 50 μg per mL of tetracycline, and nutrient agar. 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:

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. Spread plating, which was done on the last three tasks, grew bacteria that was very hard to count because of its prevalence on the plate.

A summary of the plate counts for each sample is included in the Figure below. The soil, tap water, and bioreactor yielded significantly more bacteria on all three types of plates than did any of the other treatments. However, all treatments, including breathing on a plate and coughing, resulted in some bacterial growth on the agar plates.
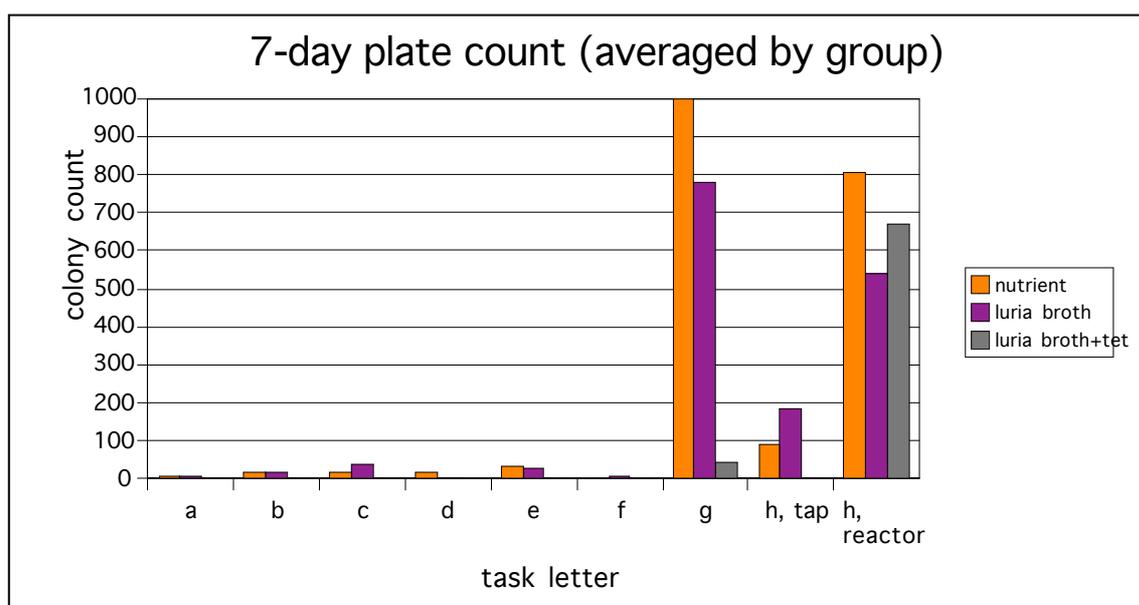
In order to access the affect of tetracycline on bacterial groth on the agar plates, a calculation of the % resistance of bacteria was performed on the last three samples (soil, tap water, and bioreactor). % resitsance equals the number of colonies counted on the tetracycline plate divided by the total colonies found on the same media, without tetracycline addition. These results are included in the table below.

Sample	LB Plates (cfu)	% Resistant Bacteria
Soil	1000	5
Tap Water	92	2
Bioreactor sample	800	124

Nine to ten times more colonies from the soil and bioreactor samples grew than from the tap water alone. However, only five percent of the bacteria from the soil sample were resistant to tetracycline. In contrast, over one hundred percent of the bacteria from the bioreactor were tetracycline-resistant. Of these samples, tap water yielded the least colonies, of which two percent were resistant.

DISCUSSION

The first several treatments (through soil) demonstrate how easy it is to culture bacteria from the environment on agar plates. None of the treatments resulted in zero growth. These results are significant since plating is one of the most basic and common of experimental methods to detect and count bacteria in environmental samples. In this experiment, agar plates were contaminated (i.e., colonies grew) when exposed to human breath only briefly. With this in mind, it is important for laboratory workers to use sterile technique when working with microorganisms in order to assure quality measurements.

In this experiment, two different agars were used. In all the treatments, colony counts from LB media and nutrient agar were different, demonstrating that the type of media influences the quantitative results. These results are consistent with the known bases of plating methods. In one study measuring the number of morphologically distinct bacteria that could be isolated from soil samples, a set of 25 different media selected for seven times more bacteria than a single media alone (Balestra and Misaghi, 1997). With this in mind, it is not surprising that LB and nutrient agar selected for different bacterial counts from the same sample.

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.

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