MICROBIAL TECHNIQUES PROCEDURE

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METHOD

Streaking: The process of streaking includes an inoculating loop, agar plates and modeled yeast plate. The inoculating loop with a small head is then flamed and cooled down. A previously modeled agar plate with yeast is then used to conduct the experiment. I touched the yeast using the inoculating loop on the agar plate with lid tilted 45 degrees, so the plate does not get contaminated. The yeast is then quickly streaked in strokes from longer to shorter in one quadrant of the plate. The inoculating loop is then flamed again and cooled down. The loop is then used to streak in the quadrant next to it. The strokes go from longer to shorter, taking the yeast from another quadrant into the new quadrant 2. The loop is then flamed again and cooled down. We streaked the same way in quadrant 3 and 4 taking the yeast from quadrant 2 and 3 respectively and flaming the loop in between. The inoculating loop was then flamed and cooled for the last time.

Spreading: The process of spreading first started with making agar plates. The agar liquid was put on a Bunsen burner, so that it doesn't harden in room temperature. The agar was poured into 4 different plates and was set aside to cool down and solidify. I used five different plastic tubes: A, B, C, D, E, on a stand for dilution process. All the tubes except A were then filled with 2.7 ml of water using a glass pipet. The pipet was flamed every single time on a burner to kill microbes. Tube A was filled with diluted yeast concentration of 1 ml. We used 1ml disposable sterile pipet to take 0.3 ml of liquid from A and transferred it to B. Then we swirled the tube B and used a different pipet of the same type and took 0.3 ml from B to C. We followed the same process and did C to D and D to E. Then we used the solidified agar plate and put it on a rotator. The inoculating loop with the bigger head was then dipped in ethanol and flamed. Then we used

micropipettes with a tip to take 0.125 ml of diluted yeast from C, D and E. I quickly poured the liquid into the agar plate and used the spreader and rotator to spread the liquid throughout the plate. This process was done with 0.125 ml from tubes D and E as well.

RESULTS

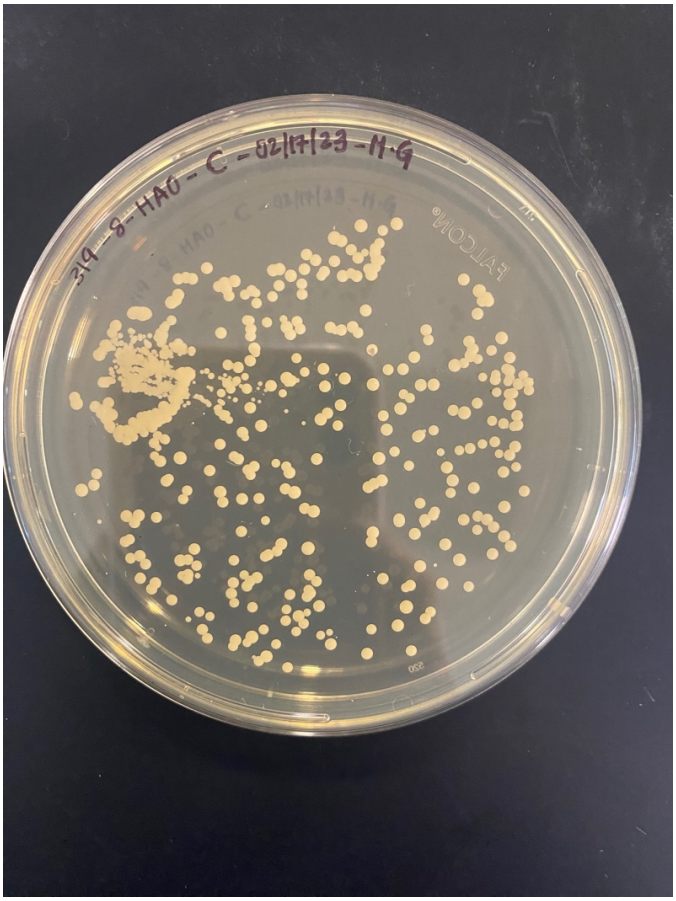

Figure 1 Spreading plate using diluted yeast from tube C.

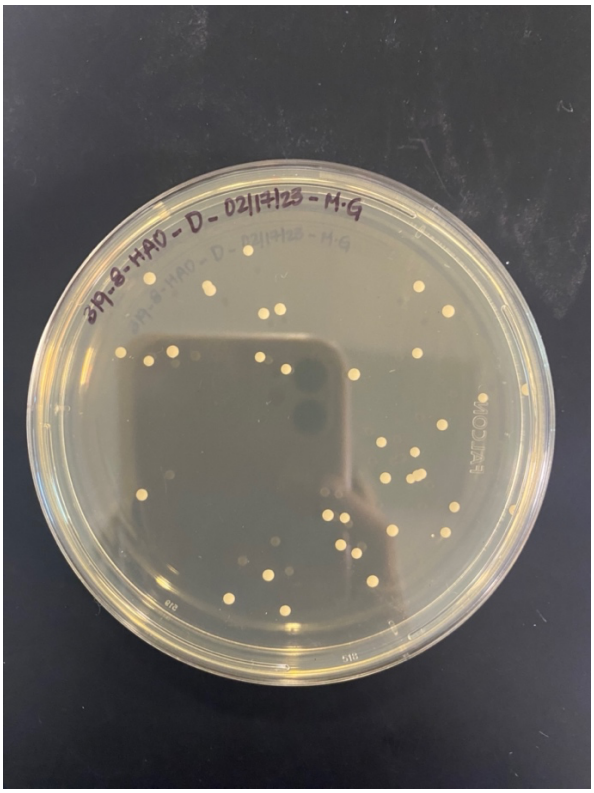

Figure 2 Spreading plate using diluted yeast from tube D.

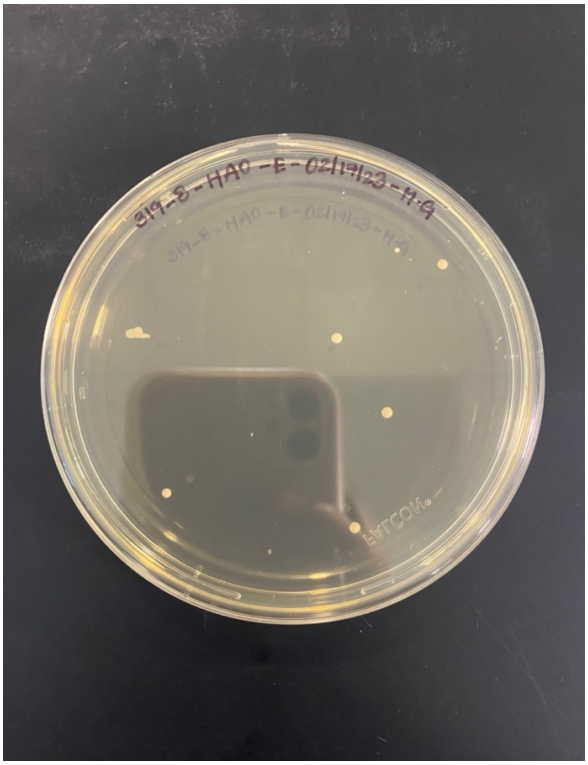

Figure 3 Spreading plate using diluted yeast from tube E.

Figure 1,2 and 3 showed that the growth pattern is proportional to the concentration of yeast because C had the highest concentration out of D and E and the number of colonies were more. We had only used C,D and E for spreading.

Tube	C	D	E
Number of colonies	310	39	6

The number of colonies in D are more concise and clearer than tube C, so we used that it finds the titers in all other tubes.
Titers in tube D:

$$\frac{39\ cells}{0.125\ ml} = \frac{312\ cells}{ml}\ \ in\ D$$

The cells were diluted in 10 X dilution, so we multiply 10 every single time we go backwards, so the titers in C, B and A are:

$$\frac{3.12*10^3\ cells}{ml}\ in\ C$$

$$= \frac{3.12*10^4\ cells}{ml}\ in\ B$$

$$= \frac{3.12*10^5\ cells}{ml}\ in\ A$$

Titer in tube E will be: $\frac{31.2\ cells}{ml}$.

DISCUSSION

The results showed that the number of colonies decrease when the solution is diluted from tube A to E. By observing the data, we knew that dilution isolates colonies based on the proportionality of dilution. The problems that might affect our results would be contamination, if the spreader wasn't flamed well or if we forget to flame it in between, this causes contamination of the agar plates. If caps or tubes are placed on the desk, contamination occurs there as well. The microbes in air and the working area will give the slightest variance in the results. The number of cells on agar plate was 39 cells. According to the titer's calculation, the number of cells in E should be 31.2 cells, while the actual is $\frac{6\ cells}{0.125\ ml} = 48\ cells/_{ml}$. This number is really close to the calculated value, so we can conclude that it is indeed 10 X dilution. The slight variance of values might be because the spreading was only done a few times which might have resulted in clumping of the solution at one place.