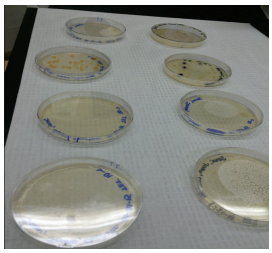
26 January 2015 – JB

Identifying Bacteria with DNA Sequences

Bacteria appear in three common shapes, Spirillum, Coccus, and Bacillus. Bacteria can be either gram-positive, meaning it has a thick layer of peptidoglycan in its cell well, or gram-negative, meaning it lacks the thick layer of peptidoglycan. Bacteria are most visible when they are in a colony, a group of a bunch of bacteria together. To determine if a bacterium is gram-positive or gram-negative, a gram stain procedure is done, and if the bacteria are purple after the procedure, then it is gram-positive. The purpose of this lab was to become more familiar with bacterial identification and to identify DNA sequences of bacteria.

In this lab we first observed both the hay infusion and the gar plates. We then created two wet mounts with two colonies from separate plates. We observed those colonies under a microscope and determined the shape and type of bacteria that they were. We determined this by looking at shape, color, motility, and size. Next we carried out the gram staining procedure with four separate colonies from four separate plates (2 from the same plates used for the wet mounts). Lastly, we ran PCR on the four colonies from the plates that we used for gram staining.

In the most recent state of the hay infusion culture, it was odorless, the water was clearer, and a majority of the dirt had settled at the bottom of the jar. I hypothesize that the bad smell from the week before had disappeared because the decomposition of organic material may have been completely consumed by bacteria.

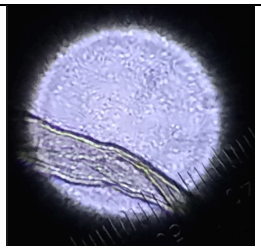


Before seeing the agar plates, I expected there to be a lot of growth on the plates with no tetracycline. I expected to see minimal to no growth on the tetracycline plates because it is an

antibiotic and would normally act to prevent growth of bacteria. Some growth may still occur because tetracycline only fights certain type of bacteria, and others that are resistant to the drug may persist and grow.

Dilution	Agar Type	Colonies Counted	Conversion Factor	Colonies/mL
10^-3	Nutrient	50	x10^3	50 x 10^3
10^-5	Nutrient	61	x10^5	61 x 10^61
10^-7	Nutrient	1	x10^7	10^7
10^-9	Nutrient	1	x10^9	10^9
10^-3	Nutrient + tet	1	x10^3	10^3
10^-5	Nutrient + tet	56	x10^5	5.6 x 10^8
10^-7	Nutrient + tet	0	x10^7	0
10^-9	Nutrient + tet	0	x10^9	0

Plate 10^-5 Nutrient plus tetracycline may have been high in bacteria population because it was a bacteria that was resistant to the drug or antibiotic. Also, contamination may have also been possible, causing the unusual number to occur. Contamination may have also occurred on plates 10^-7 nutrient and 10^-9 because the bacteria population is unusually low. Overall the tetracycline seemed to reduce bacteria growth indicating that it was a mostly effective drug in eliminating bacteria. It seems that only one species of bacteria were unaffected by the drug. I think that the bacteria that survived must have had a very gram-positive peptidoglycan wall, defending against the bacteria well. The others may have had weaker walls, succumbing the drug.

Club Rod	N + T 10^-5	 40x	Motile
Cork Screw	N 10^-5	 40x	Motile

These wet mounts showed us the shape and type of bacteria growing on the plates.

Colony Label	Plate Type	Colony Description	Cell Description	Gram + or -
10^-5	Tet	Orange, large, round, smooth	No motility, round headed, tail	+
10^-5	Nut	Purple/clear yellow, circle, smooth	Motile, ling lined, spiral	+
10^-7	Nut	1 lawn, white/tan, smooth		+
10^-3	Nut	Purple/yellow, smooth, small		+

They all have very different appearance and descriptions.

In conclusion, the bacteria must have been so different from each other since samples were taken form various parts of the hay infusion. The PCR will tell of the DNA sequencing which will indicate their genetic strengths. This will tell why some could resist the tetracycline while others clearly could not.