Antibiotic Resistance of Bacteria - Over Rx'd and Under Ed'd

Davidson County Community College, Thomasville, NC 27360

Purpose

Bacterial strains have developed resistance against antibiotics throughout the years and have been found more frequently in the environment. This experiment will provide information on the prevalence of such bacteria in the soil of randomly assigned collection coordinates within a 20-minute radius of Davidson County Community College, Thomasville, NC.

Hypothesis

Antibiotic-resistant bacteria would more likely be found in areas with a higher human and domestic/farm animal population.

Materials

Sample Collection:
- Sterile containers and gloves

Inoculating LB:
- Sterile Falcon™ tube
- DiH2O
- P200 micropipette
- Spreading rod and Bunsen burner
- LB w/AMP agar (Luria-Bertani nutrient broth w/ampicillin)
- Incubator

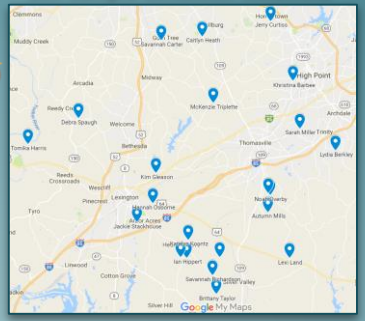
PCR Amplification:
- PCR tube: 100 µL DNAse-free H2O
- Thermocycler
- PCR tubes: (#1) 22 µL Bla-TEM and (#2) 22 µL Bla-SHV
- Mineral oil

Gel Electrophoresis:
- Agarose gel with ethidium bromide (fluorescence)
- Buffer solution
- Methylene blue dye
- 100bp DNA ladder (standard)
- Power source and UV light

Sample Collection

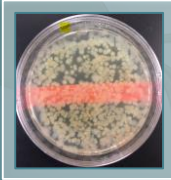
Soil samples were collected approximately 2-3 inches below the surface. Sterile containers and aseptic technique were used to prevent contamination.

Soil Sample Collection Locations



Soil Preparation and Plating

1. Soil and twice the volume of DiH2O were added to a Falcon™ tube, shaken vigorously, and allowed to settle (~10 minutes)
2. 200 µL of supernatant was removed and inoculated to LB w/AMP agar:
    1. Transfer supernatant to center of agar using P200 micropipette
    2. Pass spreading rod through Bunsen burner flame and allow to cool (briefly); gently glide spreader along agar to evenly distribute soil supernatant
3. Inoculated agars were incubated at 37ºC:



Sample #6489
Coverage: 96.46%
Colony count: 520

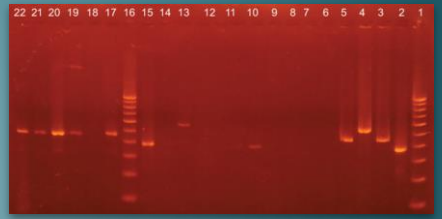
Sample #4549
Coverage: 37.48%
Colony count: 598

PCR Amplification

Polymerase Chain Reaction (PCR) is the process of (1) *Denaturing* (splitting DNA strands), (2) *Annealing* (bonding primers (Bla-SHV and Bla-TEM) to a matching DNA sequence), and (3) *Extending* (activating Taq polymerase to attach nucleotides to form an identical copy). This process can yield billions of copies of target DNA sequences to enhance detection!

Gel Electrophoresis

20 µL of Bla-TEM and Bla-SHV are pipetted into wells on agarose gel with varying pore sizes and fluorescent dye. Electric current is applied, and (-) DNA migrates toward the (+) anode side. Each size of DNA fragment migrates through the gel until it reaches a smaller pore size than itself. The larger the fragment, the shorter the distance traveled.
UV light is applied and the DNA fragments detected fluoresce orange. Bla-SHV is detected at 747 bp; Bla-TEM at 445 bp.



Gel Example: Lanes 6-9, 11, 12, 14, 18 = Negative
Lanes 2, 20, 15 = Bla-SHV (+)
Lanes 3, 5, 17, 19, 20-22 Bla-TEM (+)

Results

Unfortunately, the gel electrophoresis was inconclusive, however, results are still able to be drawn from our class colony count and data collection analysis...

Class Soil Collection - Mean Data

	Avg. % Coverage	# of Colonies
Whole Class (16)	76.73%	412.11
Agri/Crop land (10)	77.12%	345
Creek/River bank (6)	75.96%	546.33

Agri/Cropland was ~1% higher in growth coverage, however, yielded less than 200 colonies on average, which could just be attributed to the species of bacteria. But, 100% of students in the class had bountiful growth on their agar, which means everyone was able to cultivate ampicillin-resistant bacteria regardless of collection location! Now the question is, were humans the one to cause the spread of resistance, or were bacteria able to evolve resistance against each other?