The Search For Antibiotics

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BSC1005 LAB

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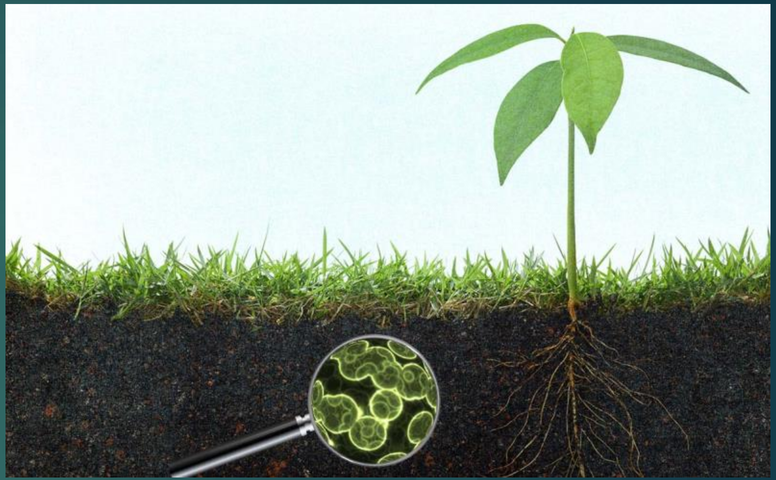
The Need for New Antibiotics

- Antibiotic crisis
  - An antibiotic is a chemical that kills bacteria.
  - Since the 1980s, there have been no new discoveries of antibiotics
  - Bacteria is becoming resistant to the antibiotics we currently have
  - In the United States, there are over 2 million drug-resistant bacterial illnesses
    - 23,000 deaths and $35 billion in economic losses per YEAR (CDC,2015)
  - "If no significant action is taken by 2050, super bugs will kill more people than cancer and diabetes COMBINED and will result in 300 million premature deaths." –World Health Organization

SWI (Small World Initiative)

- Small World Initiative
  - A program that encourages students from schools around the world to help discover new antibiotics
  - Established by Dr. Jo Handelsman in 2012
  - To date there are about 275 schools around the world participating in SWI program
  - Allows credit to go to the student if antibiotics are developed with their soil

Soil Microbes



- Soil Microbes
  - Source of 2/3 of antibiotics today, this includes: bacteria, and fungi.
  - Fun Fact: There are more microbes in a teaspoon of soil than there are people on the earth. Soils contain about 8 to 15 tons of bacteria, fungi, protozoa, nematodes, earthworms, and arthropods.

ESKAPE Pathogens

- ESKAPE pathogens
  - Bacterial infections that are resistant to antibiotics
- Safe relatives
  - Physiologically the closest bacteria to the actual ESKAPE pathogens

ESKAPE Pathogens	Corresponding Safe Relatives
Acinetobacter baumannii	*Acinetobacter baylyi*
*Enterobacter**	*Enterobacter aerogenes*
Enterococcus faecium	*Enterococcus raffinosus*
Klebsiella pneumonia	*Escherichia coli*
Pseudomonas aeruginosa	*Pseudomonas putida*
Staphylococcus aureus	*Staphylococcus epidermidis*

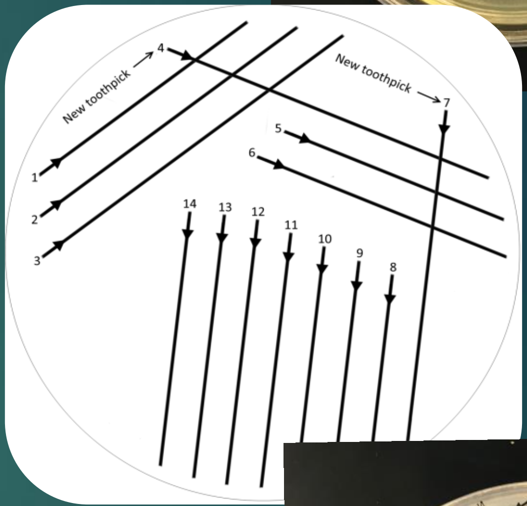
* Several strands of this bacteria are antibiotic resistant

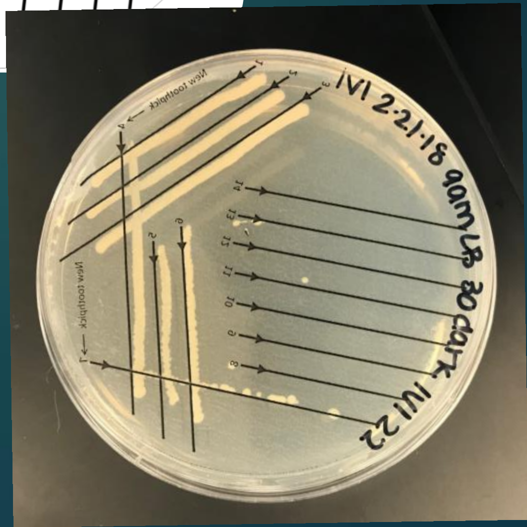
Experimental Process

- Students collect soil from locations of their choice
- Soil is diluted and bacteria is isolated and spread onto plates of food
- Colonies are patched onto new plates of food to create pure colonies
- Active isolates are picked based off zones of inhibition
- Tested bacteria to see if they kill safe relatives
- Extracted organic compounds from isolate
- Tested resistance of isolate to common antibiotics
- Characterized the isolates with antibiotic activity
- Recorded data on the SWI database

Techniques Used for Experiment

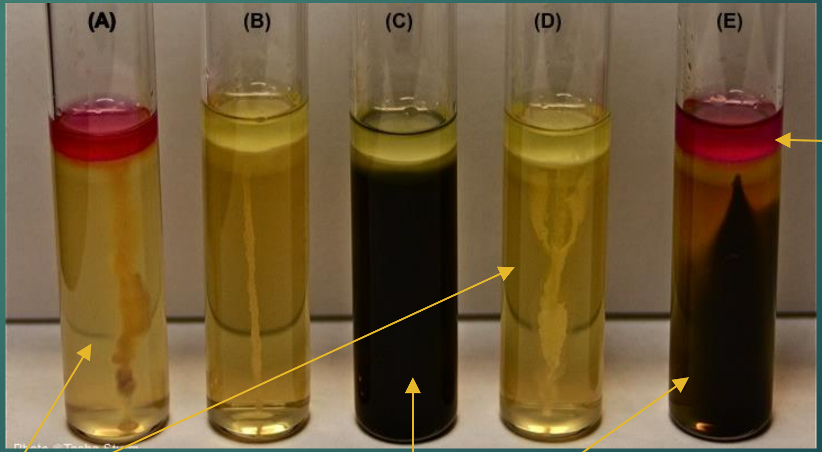
- Picking and patching
  - Isolating colonies and starting a pure culture.
  - “Pick” distinct colonies from a dilution plate.
  - “Patch” them onto a fresh plate.
  - Allows us to locate isolates that show zones of inhibition
    - Indicates that a microbe is secreting a substance that kills the safe relative
- Streaking for singles
  - Process of diluting of bacteria to obtain single colonies





Experiments Performed

- MacConkey Agar
  - Distinguishes if the bacteria produces lactose fermentation
    - Pink/red = bacteria ferments lactose
    - Tan/white= bacteria does not ferment lactose
  - Only Gram-Negative will grow on agar
- SIM Media
  - Sulfur-indole-motility
  - Distinguishes if bacteria:
    - Produces hydrogen sulfide
    - Produces indole
    - Is motile



Indicates indole production

Indicates motility

Indicates hydrogen sulfide production

Experiments Performed (cont.)

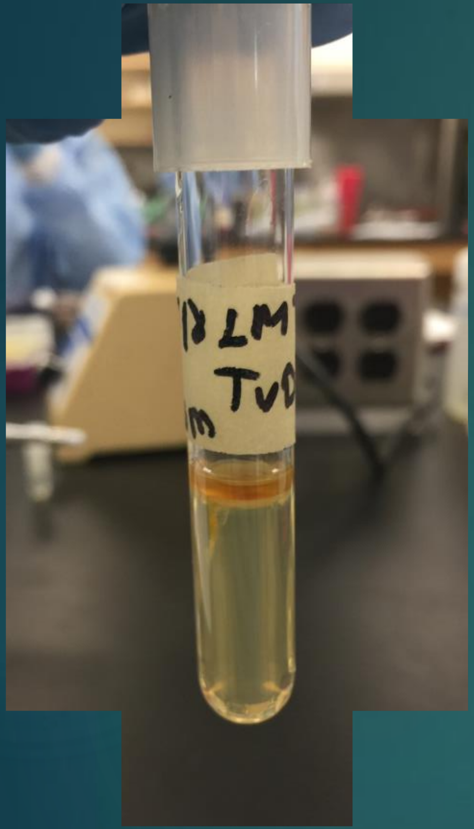
- Organic Extraction
  - Used to extract the antibiotic from the soil isolate
  - Process allows the cells to burst, hopefully releasing the antibiotic that it produces.
- Catalase
  - Checks for the presence of enzyme catalase in bacteria that hydrolyzes hydrogen peroxide
  - Bubbles will show if the bacteria posses enzyme catalase
- Agarose Gel Electrophoresis
  - Agarose- Polysaccharide made from seaweed
  - Allows DNA to pass through when placed in an electric field
  - Used to separate and see our PCR DNA fragments
- Gram staining
  - Helps one classify the bacteria as either Gram positive or Gram negative

Lunischa's Experiment

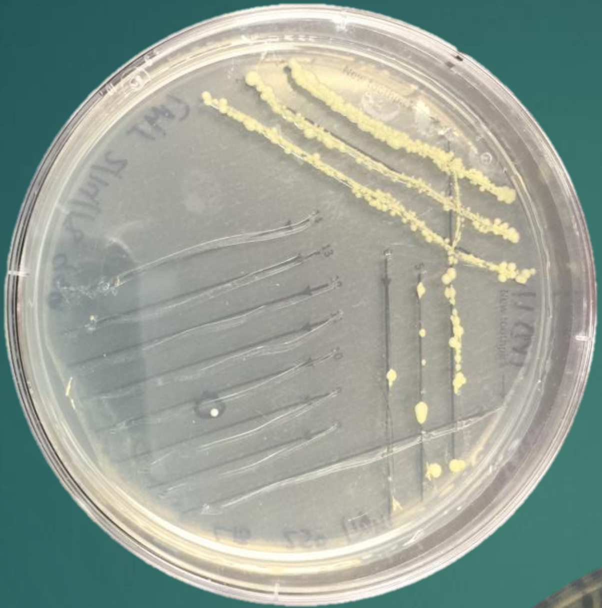
- Switched to another person's soil during the 2nd lab
- After Gram staining, bacteria appeared pink
  - Conclusion: Gram negative bacteria
- Isolate did not grow in MacConkey agar due to Gram Positive bacteria
  - Results were different than the Gram staining
  - Possibly due to old cells or different colony used
- DNA was sent to Yale

Original soil

Lunischa's Experiment (Cont.)

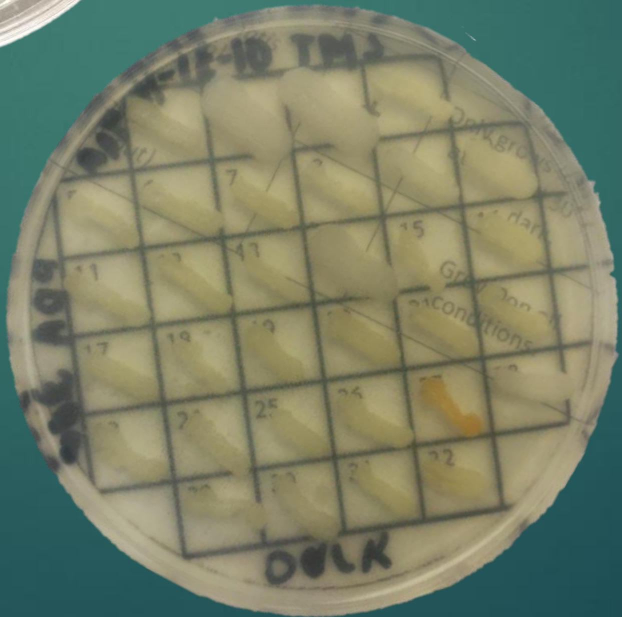


Bacteria showed signs of motility and is metabolizing tryptophan

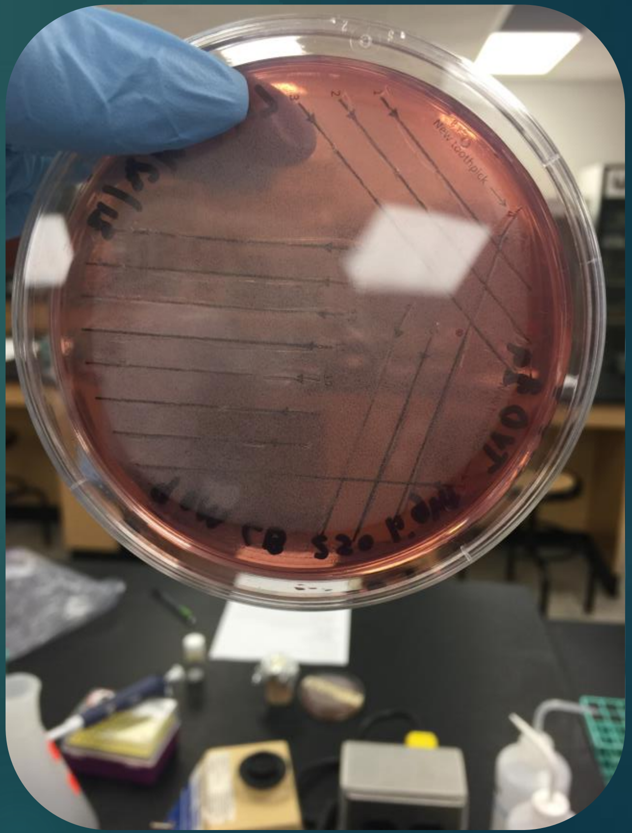


Streaking for singles

Picking and patching

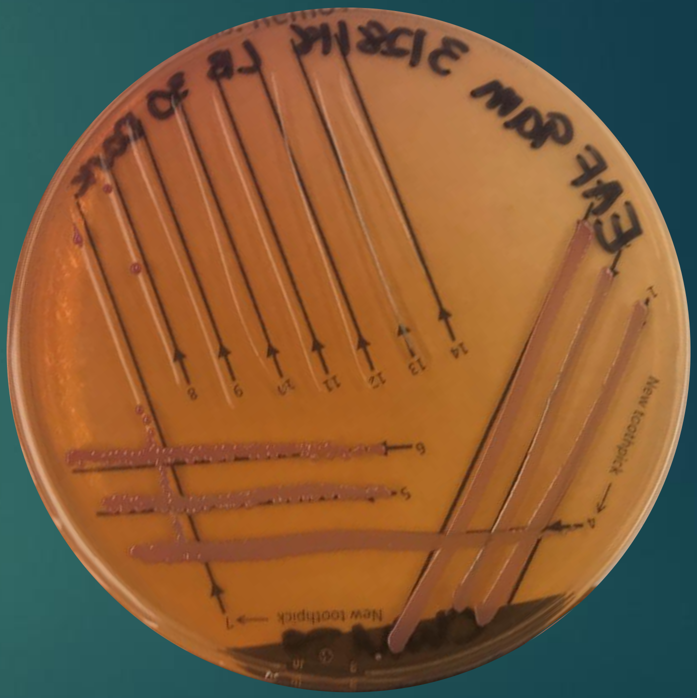


No bacterial growth on MacConkey agar



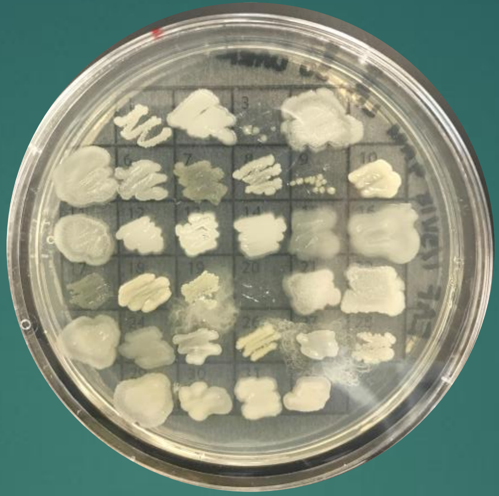
Eliana’s Experiment

- Soil was switched to someone else’s starting on the 8th lab
- After Gram staining, bacteria was purple
  - Conclusion: Gram positive bacteria
- MacConkey agar experiment resulted in bacteria fermenting lactose
  - Resulting in Gram negative bacteria
- Gram results may have been different in experiments due to old cells or different colonies used.
- 16s rRNA gene was successfully amplified using PCR
- DNA will be sent to Yale University for further research



Eliana’s Experiment (cont.)

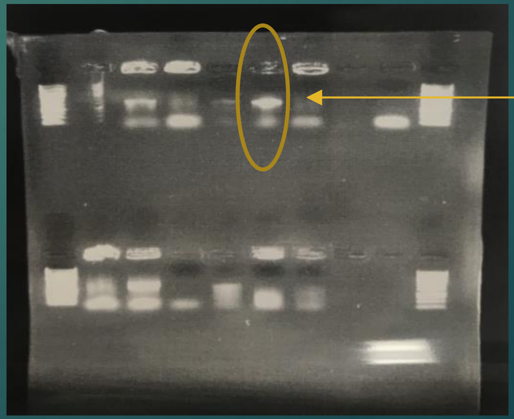
Original soil used before lab 8



Picking and patching

Streaking for single colonies

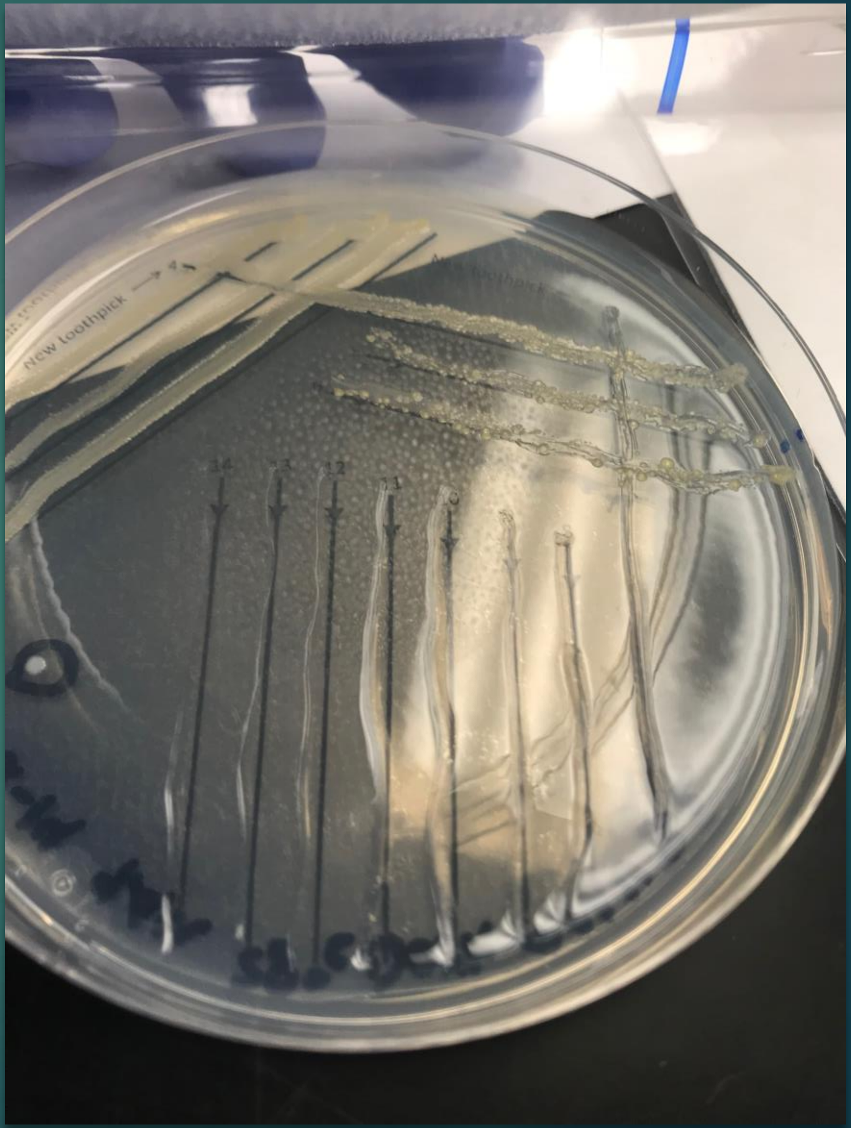
Bacteria showing motility and that it produces tryptophan



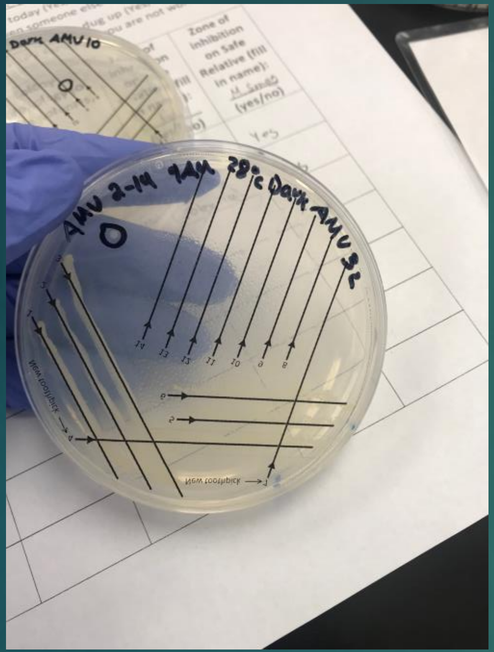
DNA will be sent to Yale!

Asley's Experiment

- Had to use Tricia's soil from the beginning
- Conditions: TSA 28° c Dark
- Safe Relatives tested against: *M. smeg* and *A. bay*
- After Gram staining Bacteria appeared pink
  - Resulting in Gram (-) bacteria
- DNA was not sent to Yale



Asley's Experiment (cont)



Streaking for singles

SIM tube shows no Motility

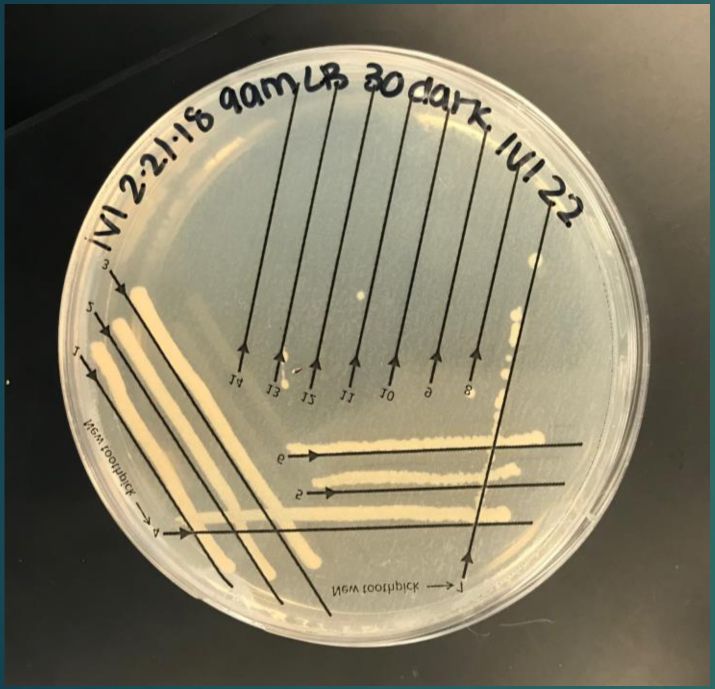
Picking and Patching

Isabella’s Experiment

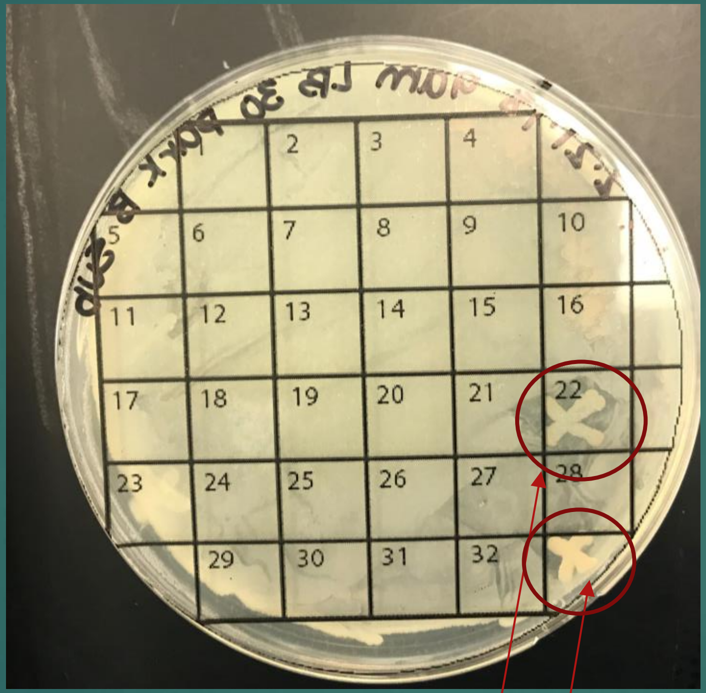
- Was able to use original soil throughout the entire experiment
- Experiment was successful and DNA will be sent to Yale for further testing
- Tested against safe relatives B. sub and A. bay
- Conditions: LB 30° Dark

1: 10,000 most successful single colony Dilution

Isabella’s Experiment (cont.)



Streaking for singles



Zones of inhibition

SIM tube showed slight motility

Student Results

Student	Own soil	GPS coordinates	CFU/g	Gram (+) or gram (-)	Single colonies	Catalase production	Tryptophan Metabolism	Motility on SIM	Lactose fermentation	Hydrogen Sulfide Production
Asley	No	**Lat** 26.401801 **Long** -80.203176	1.0*10^10	+	TMTC	Yes	No	No	No	No
Eliana	No	**Lat** 26.369014 **Long** -80.100035	TMTC	+	TMTC	Yes	Yes	Yes	Yes	No
Isabella	Yes	**Lat** 26.3684 **Long** -80.1032	1.0*10^7	-	TMTC	Yes	Yes	Yes	Yes	No
Lunischa	No	**Lat** 26.401801 **Long** 80.203176	2.4*10^7	+	7	No	Yes	Yes	Don't Know	No

Antibiotic Activity and Resistance of Our Bacterial Patches.

Student	**Medium**	**Temperature (C)**	**Light or Dark**	**Antibiotic Activity Against**	**Antibiotic resistant to**
Asley	TSA	28	Dark	none	Gram, Tet
Eliana	LB	30	Dark	*E.caro, E.raff, B.sub,*	Gram, Tri, Rif, Tet, P/S
Isabella	LB	30	Dark	*E.caro, B.sub*	Gram, Tri, Rif, Tet, P/S
Lunischa	LB	25	Light	*P.put*	Gram

Antibiotic Activity Results of our Bacterial Organic Extracts

student	kills *S.epi*	Kills *E.coli*	Kills *E.caro*	Kills *E.raff*	Kills *A.bay*	Kills *B.sub*	Kills *E.aero*	Kills *M.smeg*	Kills *P.put*
Asley						Yes			
Eliana						Yes			
Isabella						Yes			
Lunischa									Yes

DNA sequence results

	Asley	Eliana	Isabella	Lunischa
PCR product and sequence	267-JB31-S18-SWI27FHT	138-EAF-SWI27FHT	257-JB13-F17-SWI27FHT	150-LMT-SWI27FHT
Organism with closest match to DNA sequence	*Bacillus* sp.2059 16S ribosomal RNA gene	*Pseudomonas* putida strain N1R genome assembly, chromosome: I	*Chryseobacterium* kwangyangense strain Cb 16S ribosomal RNA gene, partial sequence	*Bacillus* aryabhattai strain L14 16S ribosomal RNA gene, partial sequence
Percent identity	97%	99%	99%	94%

Conclusion/Now What?

- Our DNA was frozen in a glycerol stock at -80 °C
- Our bacteria produced chemicals that can kill safe relatives
- Everyone’s DNA except for Asley’s was sent to Yale for further research
- Hopefully our DNA will help produce antibiotics in the future

Discussion/What We Learned

- We developed more of an understanding of bacteria and their growth/ mutation
- We developed more of an understanding of the scientific process and collaboration with others
- SWI has:
  - Exposed us to real life scientific experimentation
  - Opened our minds to consider careers in science
  - Introduced us to the importance of finding new antibiotics and preventing the spread of bacterial illnesses