Bugs That Make Drugs: The Investigation of Antibiotic Producing Bacteria from Local Fairfield University Soils

Fairfield University BASE Camp

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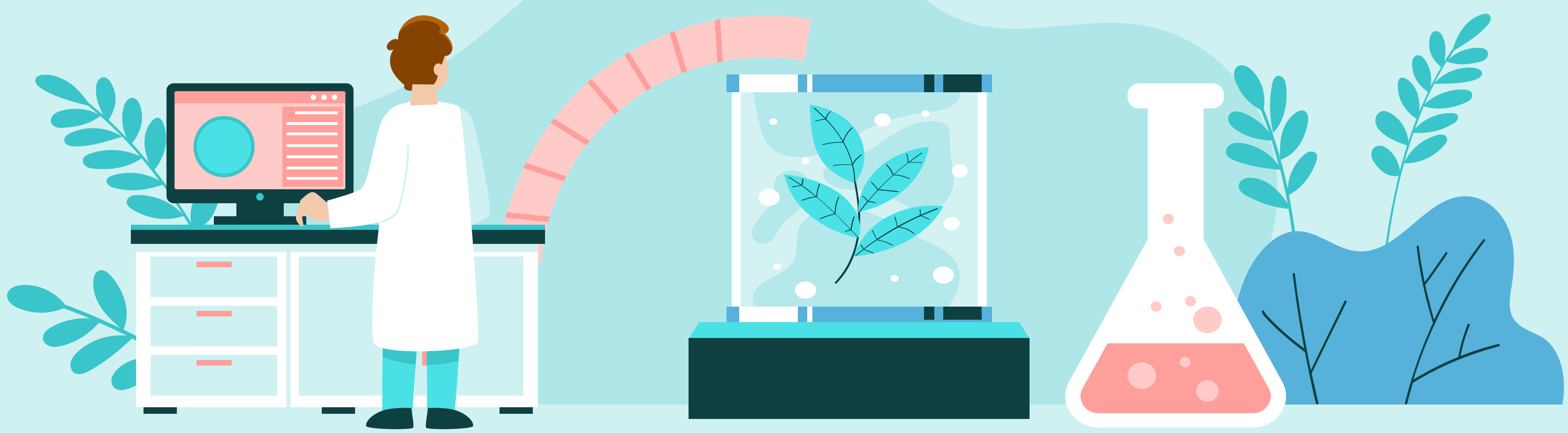
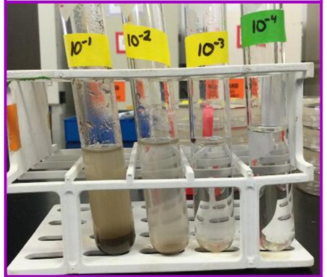


Introduction

- Soil is a habitat for a multitude of bacteria, fungi, and protozoa. Within one gram of soil, there can be as many as a billion bacterial cells.
- Bacteria can be integral to human health due to its antibiotic qualities.
- However, due to the overuse of antibiotics in the farming industry and healthcare, antibiotic resistance has increased worldwide. As a result, microbiologists are attempting to discover novel bacteria that exhibit antibiotic behavior.
- Approximately 35,000 people die from antibiotic resistant infections annually in the United States (CDC 2019).
- Tiny Earth is an organization that involves students across the world to fight against antibiotic resistant bacteria.
- As a Tiny Earth partner, the objective of our research was to screen soil samples for antibiotic-producing bacteria.

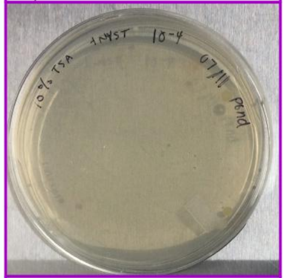
The Overview of Methods and Procedure

1)



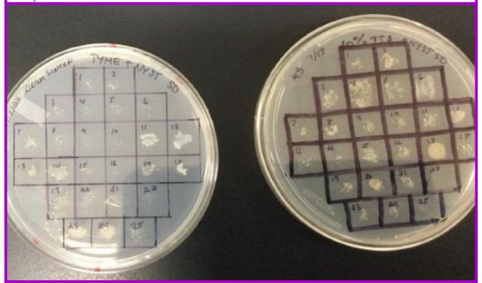
1 gram of each soil sample was serially diluted resulting in a 10-fold dilution of the soil sample

2)



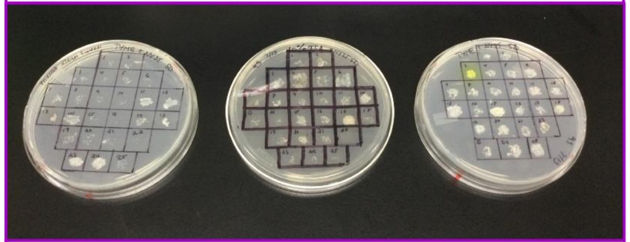
Each dilution was cultured on two types of nutrient media to aid in growth (TSA 10% and TYME)

3)



Inoculated colonies from cultured dilutions to prepare master plates

4)



Tested master plate soil isolates for antibacterial activity against select ESKAPE strains and *Staphylococcus aureus*

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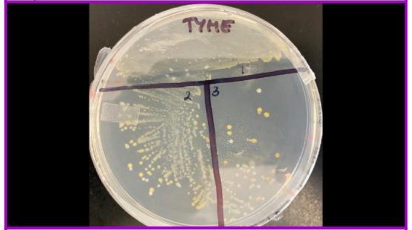


Soil isolates exhibiting antibiotic activity were streak plated for pure culture isolation

Results: Serial Dilution of Soil Bacteria

10^{-2}

10^{-3}

10^{-4}

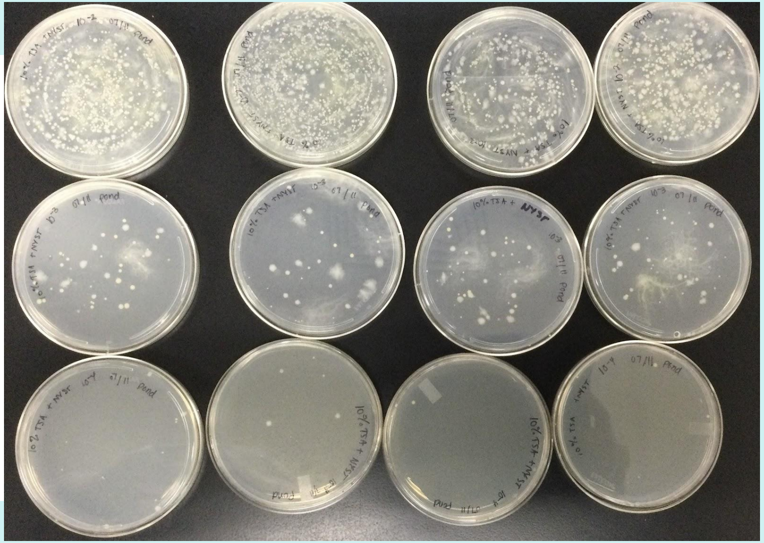


Figure 1: Growth on serial dilution plates after 48 hours of incubation at 32˚C

Results: The first row of plates (which were diluted to the 10^{-2}) were full of bacteria. The last row of plates were diluted to the 10^{-4} power, and had little to no bacteria growth. This shows that as we diluted the soil the less bacteria was observed.

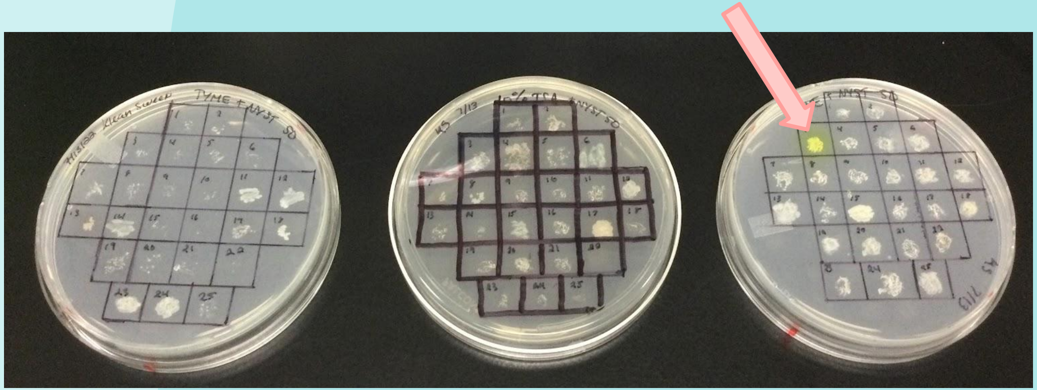


Figure 2: Serial dilution plates were used to isolate 25 distinct colonies on each plate, creating 6 master plates

Results: We observed bacteria colonies had diverse morphologies and some were pigmented as shown in the figure.

Results: Colony Count of Serial Dilution Plates

Table 1: Colony Counts of Total Bacteria on TYME

	Kleen Sweep			Pond		
Dilution	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Vol. Plated	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Number of Colones Counted	TNTC	156	80	TNTC	48	15.5
CFU*/mL	TNTC	1.56×10^{6}	8×10^{6}	TNTC	4.8×10^{6}	1.55×10^{6}

Table 2: Colony Counts of Total Bacteria on 10% TSA

	Kleen Sweep			Pond		
Dilution	10^{-2}	10^{-3}	10^{-4}	10^{-2}	10^{-3}	10^{-4}
Vol. Plated	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Number of Colones Counted	TNTC	181	25	TNTC	123	13
CFU*/mL	TNTC	1.81×10^{6}	2.5×10^{6}	TNTC	1.23×10^{6}	1.3×10^{6}

Results: ESKAPE Screening

ESKAPE pathogens are able to resist known antibiotics and are the most prominent nosocomial infections. They are able to avoid antibiotics and can be quite resistant. We performed an ESKAPE screening of our soil isolates to test for antibacterial activity against the ESKAPE strains.

Enterococcus *faecium*

Staphylococcus *aureus*

Klebsiella *pneumoniae*

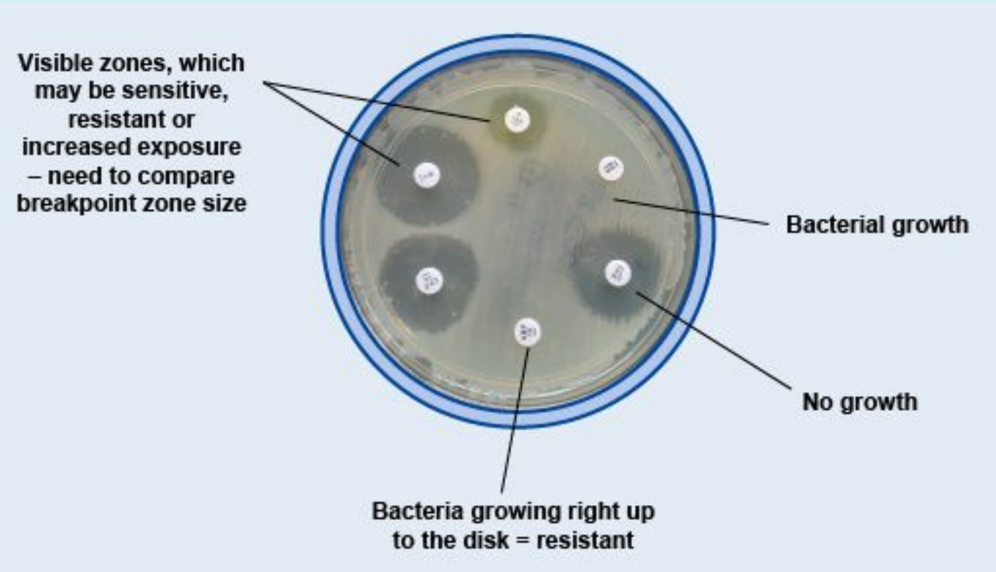
Acinetobacter *baumannii*

Pseudomonas *aeruginosa*

Enterobacter *species*

ESKAPE Screening: Zone of Inhibition

In order to perform the ESKAPE screening, we inoculated agar plates with either S. *aureus* and A. *baylyi*., and added our previously grown, diverse bacterial colonies to the plate. We then observed the plates, taking note of any **zones of inhibition**. A **zone of inhibition** is an area on the agar plate where the ESKAPE bacteria could not proliferate because of the presence of antibiotics.



Results: ESKAPE Screening of selected Bacterial Isolates

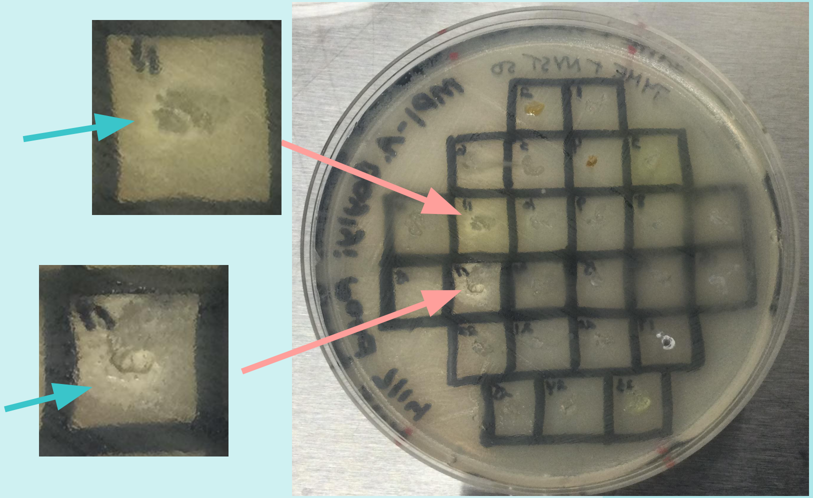


Figure 3: Master plate of bacterial colonies grown on TYME media in the presence of A. *baylyi*.

Results: In some bacteria, zones of inhibitions were created show the antibiotic activity towards S. *aureus* and A. *baylyi*. For example, image 2 box 11 contains a zone of inhibition.

Results: T-Streak

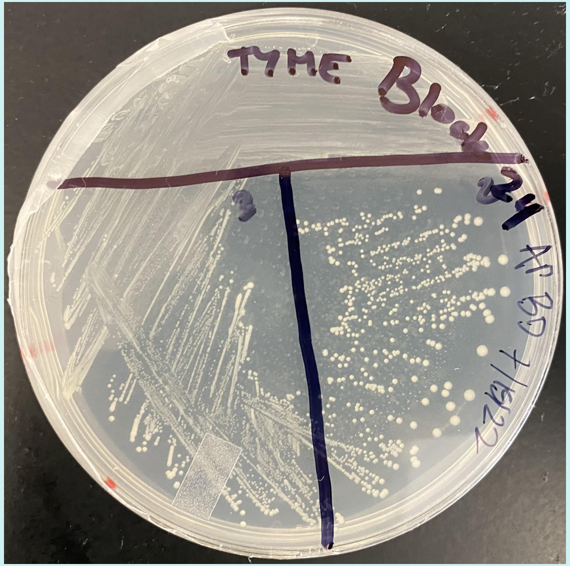


Figure 4: Bacteria taken from “Kleen Sweep” grown as a pure antibiotic producer

Results: An isolate from the Kleen Sweep soil sample with activity against S. aureus was T-streak plated onto TYME media. Isolated colonies will be used in future studies.

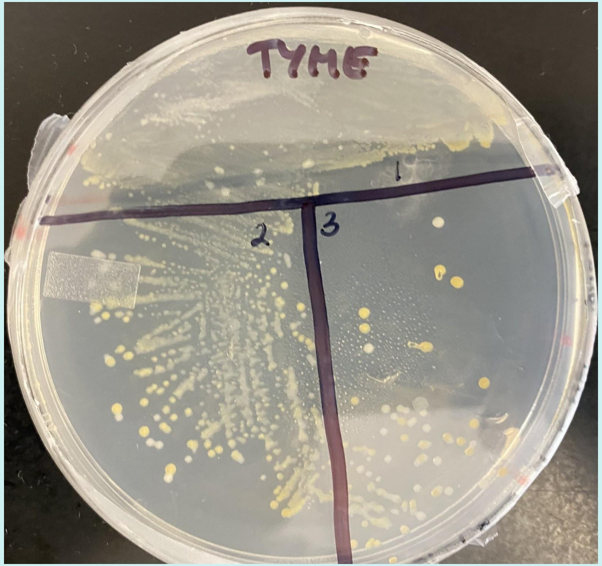


Figure 5: Bacteria taken from “Pond”

Results: The T streak above contains multiple colors, yellow and white, of bacteria. The multitude of colors shows that the isolate is not pure. Additional streak plating is needed to obtain a pure culture of this isolate.

Discussion

- Antibiotics are a lifesaving treatment for some illnesses, but due to its overuse there is a great need for discovery of new antibiotics.
- We are among thousands of students around the globe who've joined the cause to find novel antibiotics to address the antibiotic resistance crisis.
- In this study, we demonstrated that antibiotic-producing bacteria can be easily found in soils on the campus of Fairfield University
- In order to further continue this research, the correct genus and species of the antibiotic-producers should be identified along with the biochemical properties of the antibiotic metabolites.

Acknowledgements

Fairfield University

BASE Camp

Tiny Earth

https://tinyearth.wisc.edu/

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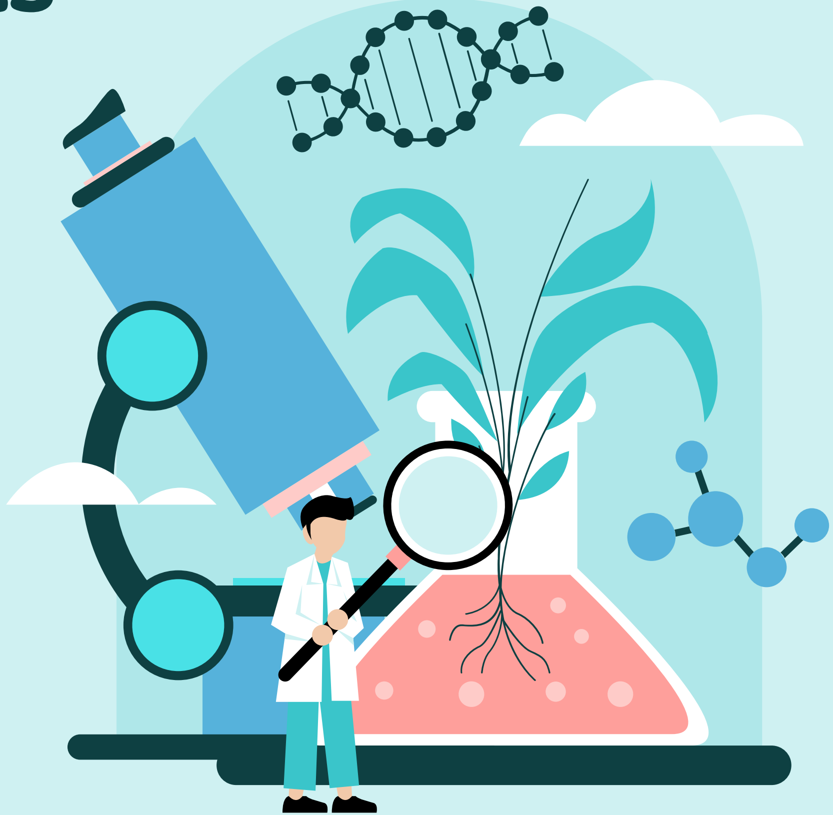

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