

Tiny Earth: Reverse Antibiosis Approach

Lucas Joseph, Casey M. Finnerty, Ph. D.
Biology Department, Winona State University, Winona, MN 55987

Abstract

Of the estimated one billion species of bacteria out there, we've only named thirty thousand of them. That's only 0.00003% percent! Despite their reputation for getting us sick, bacteria, ironically, also help keep us healthy in many different ways. Antibiotics were actually discovered by studying specific bacteria that have the capacity to repel other species of bacteria. The growing threat of drug resistant bacteria is global, and the production of new antibiotics is limited. This study aims to explore the efficiency of a method for plating and testing for antibiotic producers used by high school students in MicroMundo Albacete 2020 as well as experienced UCLM researchers. Here, after being treated with gram-negative antibiotics, the inoculated medium is flipped over like a pancake in its dish. The now new top of the medium is treated with a gram-positive bacterial tester ESKAPE strain known to be pathogenic to humans. Data is currently being collected for review.

Introduction

What are antibiotics?

- Antibiotics are drugs used to slow or prevent the growth of bacteria
- The improper use of antibiotics in the clinical field has led to a growing threat of bacteria resistant to all forms of known antibiotics
- Bacteria known to be resistant to antibiotics are known as ESKAPE strains

How do we discover new antibiotics?

- Pharmaceutical antibiotics are discovered through observation of bacterial colonies
- Bacteria use their own self made antibiotics to inhibit the growth of other bacteria around them
- Most known antibiotics come from an order of bacteria called actinomycetes

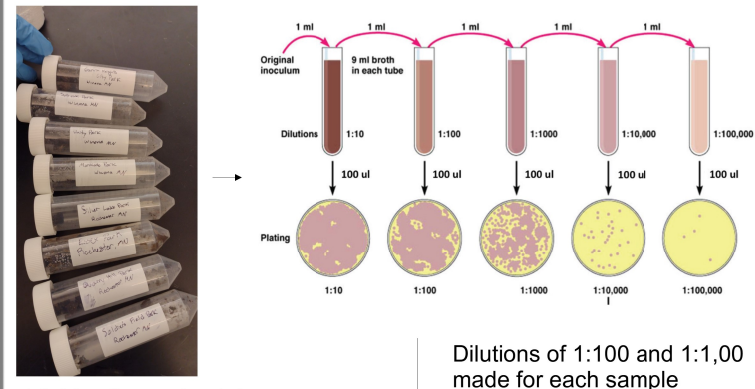
Why do this research?

- Antibiotic resistant super bugs have become increasingly fatal as we depend more and more on the same handful of antibiotics
- Increasing the variety of our antibiotic options will help decrease the prevalence of super bugs
- Flipping the agar after inoculation has been shown to improve readability of producers

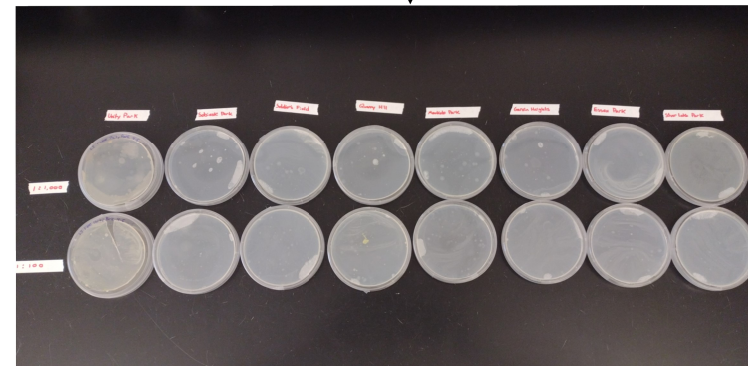
Acknowledgments

L. J. and C. M. F. would like to thank the Research and Creative Project grant committee for their contribution to the project, of which they budgeted for materials and tools that made this project possible.

Methods



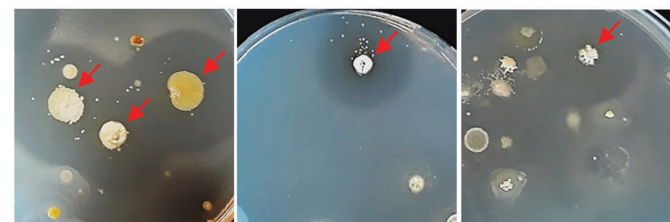
Eight soil samples taken from across Minnesota



100uL of each dilution plated onto cycloheximide infused R2A

Each agar is flipped over in its plate, inoculated side down

Potential antibiotic producers are transferred and grown on separate plates against ESKAPE tester strains



Antibiotic producers are observed by their zones of inhibition

References

- McEwen, Scott A., and Peter J. Collignon. 2018. "Antimicrobial Resistance: A One Health Perspective." Edited by Frank Møller Aarestrup, Stefan Schwarz, Jianzhong Shen, and Lina Cavaco. *Microbiology Spectrum* 6 (2). <https://doi.org/10.1128/microbiolspec.arba-0009-2017>.
- Nelson, Richard E, Kelly M Hatfield, Hannah Wolford, Matthew H Samore, R Douglas Scott, Sujana C Reddy, Babatunde Olubajo, Prbasaj Paul, John A Jernigan, and James Baggs. 2021. "National Estimates of Healthcare Costs Associated with Multidrug-Resistant Bacterial Infections among Hospitalized Patients in the United States." *Clinical Infectious Diseases* 72 (Supplement_1): S17-26. <https://doi.org/10.1093/cid/ciaa1581>.
- Smyth, Davida, Paola Sperandio, María Alvarado, Pilar Clemente-Casares, Diego Moreno, and Piet De Groot. 2020. "Article 577550 Manoj Kumar Solanki." *Frontiers in Microbiology* | www.frontiersin.org 11. <https://doi.org/10.3389/fmicb.2020.577550>

Data

Location	Garvin Heights	Sobieski Park	Mankato Park	Unity Park	Quarry Hill	Soldier's Field	Silver Lake Park	Essex Park
# Producers								
Total colonies per plate								
Hit Rate								
Latitude/ Longitude	44.033018 6 (North), - 91.650148 3 (West)	44.0411° N, 91.6143° W	44.0342° N, 91.6281° W	31.5088° N, 82.8499° W	44.0297° N, 92.4298° W	44° 0' 55.602" N and 92° 28' 2.442" W	44.0319° N, 92.4590° W	44.0780° N, 92.4760° W
Date collected	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022	January 26 th , 2022
Temperature	7°C	7°C	7°C	7°C	5°C	5°C	5°C	5°C

Project is still underway and thus not all data is complete. This table represents both data that has been and still has yet to be collected.

Discussion

• Limitations for this project included:

- Budget, as ESKAPE relative strains are not cheap
- Time, as the procedure requires twenty days of growing colonies, among other steps
- Procedure, as the agar flipping technique is not traditional and agar transfer requires tools we didn't have
- **Future improvements on this process could be:**
 - Refinement of the agar flipping and transferring techniques with clear steps and required tools
 - Use of more large 150mm agar plates to grow a larger variety of potential producers at a time

• The goal of this project is to find new and useful antibiotic producing bacteria, especially those that can inhibit the growth of ESKAPE relatives.