tiny earth

12|11|2023

in TITLE TOWN

LAMBEAU FIELD, GREEN BAY, WI

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Tiny Earth inspires and retains students in the sciences while addressing one of the most pressing global health challenges of our century—the diminishing supply of effective antibiotics. An innovative program spanning 30 countries, 47 states, Washington DC, and Puerto Rico, Tiny Earth brings together more than 14,000 students per year who are contributing to studentsource new antibiotic discovery from soil. Tiny Earth is expanding rapidly throughout Wisconsin. To date, 26 Wisconsin colleges, universities, and high schools have partnered with Tiny Earth to identify new life-saving antibiotics produced by soil bacteria, which have historically proven to be the most productive source of new antibiotics.

SCHEDULE OF EVENTS

2:30 p.m. » Registration and Poster Set-Up Begins

5 p.m. » Symposium Opens/Welcome, **Brian Merkel**

» **Michael Alexander**
Chancellor, UW-Green Bay

» **Christopher Caldwell**
President, College of Menominee Nation

» **Laurie Joyner**
President, Saint Norbert College

» **Kristen Raney**
President, NWTC

» Land Acknowledgement
Sonny Pamonicutt
Student, College of Menominee Nation

» History of Tiny Earth
Angelo Kolokithas
Professor of Microbiology, NWTC

» Antibiotic Stewardship and Innovation
Ashok Rai, MD
President and Chief Executive Officer,
Internal Medicine Physician, Prevea Health

6:15 p.m. » Student Poster Presentations

7:20 p.m. » Closing Remarks, **Brian Merkel**

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SPEAKERS

Michael Alexander

Chancellor, UW-Green Bay

Since being named seventh chancellor of UW-Green Bay in May of 2020, Chancellor Alexander initiated six strategic priorities to support the future of the University. One of those priorities is to renew and strengthen our commitment to sustainable practices and environmental stewardship. Dr. Alexander served as provost and vice chancellor for academic affairs from 2019–2020. During that time, he created an Office of Sustainability to improve efficiencies and increase the profile of UW-Green Bay as a campus traditionally engaged with environmental study; and restructured Graduate Studies and the Office of Grants and Research, setting the stage for the University's growing research efforts. Dr. Alexander has degrees from the University of Georgia, UW-Milwaukee, and UW-Madison.

Christopher Caldwell

President, College of Menominee Nation

Christopher Caldwell, President of the College of Menominee Nation, is an enrolled member of the Menominee Indian Tribe of Wisconsin. He has led the College since February 2020, serving first as Interim President, and was officially elected by the Board of Directors in June 2021.

Caldwell is the fourth person to lead CMN. He has been in a range of positions at the College including student, director, adjunct, and President. An alumnus of the College, Caldwell began his higher education here at CMN earning his Associate's Degree in Sustainable Development. He holds a Bachelor's Degree in Natural Resources from the University of Wisconsin-Madison, a Master's Degree in Environmental Science and Policy from UW-Green Bay, and is currently a Ph.D. candidate in Environment and Resources from UW-Madison Nelson Institute.

Sustainability is true to Caldwell's core having served in previous positions of; Tribal Resources Director/Compliance, Enforcement Officer for the Menominee Indian Tribe, Forest Products Technician with the USDA Forest Service's Forest Products Laboratory in Madison, student/intern with the U.S. Department of Interior Bureau of Indian Affairs-NCCE, Timber Market/Forestry Technician with Menominee Tribal Enterprises and the Director of the Sustainable Development Institute at CMN.

Laurie Joyner

President, Saint Norbert College

A noted leader in Catholic higher education, Dr. Joyner brings a wealth of experience to our community. She comes to St. Norbert from St. Xavier University in Chicago, where she has served as president since 2017. Prior to St. Xavier, she served as president of Wittenberg University in Ohio and in multiple vice presidential and dean roles at Rollins College in Florida. Dr. Joyner also was recently elected to the board of the Association of Catholic Colleges & Universities (ACCU).

She earned her doctoral and master's degrees from Tulane University, and graduated magna cum laude with a bachelor's degree in sociology from Loyola University New Orleans, where she later served on the faculty and held administrative positions of increasing responsibility.

In addition to her numerous published works, Dr. Joyner has participated as a keynote speaker and expert panelist for national conferences on a range of subjects, including higher education trends and challenges, leadership development, nonprofit governance and effective university-community partnerships.

Kristen Raney

President, NWTC

Dr. Kristen Raney serves as the president of NWTC. Dr. Raney has advanced student success and equity in technical and community colleges for more than twenty years. She has held multiple positions in both academic and student affairs, including faculty, dean, vice president, and vice chancellor. She participated in the Aspen Fellowship for Rising Future Presidents in 2021 and serves as a Peer Reviewer with the Higher Learning Commission. Dr. Raney earned a doctorate of education in higher education and leadership studies from Edgewood College; a master of science in education from the University of Wisconsin-Stout; and a bachelor of arts in English from St. Cloud State University.

Sonny Pamonicutt

Student, College of Menominee Nation

Sonny is currently a Sophomore at the College of Menominee Nation, pursuing an Associate Degree in Biological and Physical Sciences. Sonny plans to further his education with the University of WI-Madison to pursue a major in Biology or Biomedical Engineering. His intensions are to continue to work in the Biotech industry assisting with Bioprocessing, Pharmaceuticals, or Biomedical research.

Ashok Rai, MD

President and Chief Executive Officer
Internal Medicine Physician
Prevea Health

Dr. Ashok Rai is the President and Chief Executive Officer of Prevea Health – a physician-led, physician-owned multispecialty health care provider working in partnership with Hospital Sisters Health System (HSHS) hospitals across Wisconsin. Prevea Health was founded in Green Bay, Wis. in 1996, and today employs more than 2,400 people, including more than 500 providers and advanced practice providers. It offers high-quality patient care in more than 30 communities across Wisconsin, as well as a health insurance plan (Prevea360) with one of the largest coordinated networks across the state.

Dr. Rai was born and raised in Michigan. He attended undergraduate school and enrolled in the "Target M.D." program at the University of Wisconsin—Milwaukee. He completed his medical degree at the Medical College of Wisconsin, and then a residency in Internal Medicine and Pediatrics through Michigan State University at the Kalamazoo Center for Medical Studies. Following residency, Dr. Rai specialized in hospital medicine and held various leadership positions at Prevea Health before becoming the President and CEO of Prevea Health in 2009. He also continues to practice medicine, caring for patients at Woodside Lutheran Nursing Home in Green Bay and in occasional hospitalist shifts at local HSHS hospitals. Dr. Rai lives in Green Bay with his wife, Brooke. He is a proud father of three boys and three girls, and their dog, Josie.

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STUDENT POSTERS

1 Exploring River Ecosystems and Industrial Influence: A Novel Source of Antibiotic-Producing Bacteria for Combating Antibiotic Resistance

ARIEL VAUTHIER
Northeast Wisconsin Technical College

Antibiotic resistance is a pressing global health crisis, resulting from the misuse and overuse of antibiotics, threatening public health. Recognizing the need for novel antibiotics, this study explores the potential of rivers, dynamic ecosystems housing diverse microbial communities, as a source of antibiotic-producing organisms. The choice of the sampling site, near Waupaca Foundry, a major supplier of gray iron foundry in the world, was motivated by non-indigenous materials and chemicals that may influence the local environment, prompting unique microbial adaptations. Following the Tiny Earth Project methodology soil isolation was conducted using a serial dilution approach comparable to a titer method. Twenty unique bacterial colonies were chosen for further analysis using *Erwinia carotovora* and *Staphylococcus epidermidis* as test organisms. Colonies exhibiting antibiotic-producing potential were identified through their clearing on LB medium. A single antibiotic-producing colony displaying a broad antibiotic spectrum was selected for subsequent testing against eight different ESKAPE bacteria. The results of these experiments indicated that the soil from the river ecosystem in Riverview Park did yield bacterial colonies with potential antibiotic-producing capabilities due to the clearing around all eight ESKAPE bacteria. The experiments completed thus far support the hypothesis that soil from a river ecosystem possesses the capability to produce antibiotic-producing organisms. Further research is needed to identify and characterize the specific antibiotic compounds produced and to assess their potential for medical applications.

2 Rising Against The Resistance: Testing Soil Bacteria from a Chicken Coop, Woodland Dunes and Bubolz

SYDNEY HARVATH, ALEXIS SCHMIDT, SHAWNTEL HOFFMAN
Northeast Wisconsin Technical College

Scientists have been in competition with antibiotic-resistant pathogens for some time now. The negative effects of this problem are being felt not just in our own backyards but globally. As a result, students at NWTC have set out in the hopes of discovering new bacteria containing antibiotic properties. To diversify our findings, we collected soil samples from the three following locations: Bubolz in Appleton, Woodland Dunes in Two Rivers, and a chicken coop located at a private residence in Pulaski. Our hypothesis was that the chicken coop soil would contain more microbes producing antibiotic properties due to the environment abounding with bacteria from the poultry. We diluted and plated samples and chose one bacteria to continue with. The microbes found were then inoculated and examined against both *S. epidermidis* and *Erwinia* to see which microbes would produce antibiotics against the bacteria. None of our samples generated any antibiotic-producing microbes. However, proceeding with our experiment we selected one culture and examined it against relatives of the ESKAPE pathogens. Our results revealed that the bacteria from Woodland Dunes showed activity against four of the pathogens, *S. epidermidis, E. coli, E. carotovora,* and *M. smegmatis*. Our hypothesis was incorrect. The chicken coop cultivated no microbes with antibiotic properties. Our research into the properties of this soil is still proceeding. Consequently, some results are yet pending.

3 Antibiotic Producers Collected from Vivid Orthodontics and Bright Orthodontics

CHRISTINA SEERING, CAITLYN CLARK
Northeast Wisconsin Technical College

The discovery and distribution of antibiotics to the population led to better outcomes for patients suffering from disease. However, some people don't finish the full dose of the antibiotic, which allows bacteria to become antibiotic-resistant. We were presented with the opportunity by the Tiny Earth Organization to research soil. The soil we chose to research were competing Orthodontic offices: Bright Orthodontics in Oshkosh, WI and Vivid Orthodontics in Appleton, WI. Our hypothesis was that Vivid Orthodontics would have more antibiotic-producing organisms than Bright Orthodontics. We believe this because Vivid Orthodontics in Appleton, WI is in a more populated city than Bright Orthodontics in Oshkosh, WI. We diluted and plated our soil sample with the streaking method to see how many unique colonies each orthodontic office would produce. Next, we took our samples and tested them against *Erwinia* and *S. epidermidis* to determine which microorganisms produce antibiotics against bacteria. The results showed the Bright Orthodontics had three potential antibiotic-releasing microbes and Vivid Orthodontics had two. We took one antibiotic producer from each sample and tested it against the ESKAPE pathogens. Our findings showed that the selected bacteria from both orthodontics offices have produced antibiotics against two ESKAPE pathogens. Our hypothesis was incorrect; however, our research is still ongoing and results will be determined at a later date.

4 Digging Deeper: Antibiotic Producers from Soil

MADDELYN KURZYNSKE, JASMINE DOWNEY
Northeast Wisconsin Technical College

Antibiotic resistance is a big issue in the healthcare systems due to misuse of antibiotics. The Tiny Earth Project was made for students to do research on soil that may have an antibiotic-producing bacteria. Students all over the world are participating in Tiny Earth research to help test soil for bacteria with antibiotic activity. We took two soil samples from two different wetland areas. One sample came from next to a man-made pond in Clintonville, WI and the other sample was from a side bank near the Kaukauna River. Our hypothesis was that due to the number of organisms within water sources, the samples would have plenty of different bacteria and would likely have at least a few antibiotic-producing bacteria between the two sample sites. Throughout our lab we used dilution methods to seclude the bacteria within the samples. We then created independent colonies of the different types of bacteria and tested them against relatives of the ESKAPE pathogens such as *E. coli* to recognize if there was antibiotic activity being produced by our sample bacteria. When it came to our findings and results, our bacteria did not produce any antibiotic activity. Even though our hypothesis was not accurate, we have learned that the number of organisms or bacteria in an area doesn't mean you will have antibiotic-producing activity. Our experiment is still ongoing, and more results will be available soon.

5 Reaching the Post Antibiotic Era

PAIGE MUELLER
Northeast Wisconsin Technical College

When antibiotics are taken unnecessarily or when instructions are not followed, antibiotic resistance can occur, meaning they will not be as effective. This is detrimental to society because common infections we once had control over can become deadly again and unfortunately, there has not been a new antibiotic released in decades. The Tiny Earth Project is a student research project to help discover new antibiotics to combat this complex issue. I have taken soil from a well-used picnic area at my apartment due to the amount of people and food consistently present in this space. I believe there will be at least one microbe that can produce antibiotics in this area. Through different testing methods, I determined information on the specific microbe I found. My results show that I had one antibiotic producer, with it being a dual producer; it produced antibiotics against gram-negative and gram-positive bacteria. Further tests show that the microbe has activity primarily against gram-positive bacteria. So far, my hypothesis has been correct since I thought there would be one or more microbes with antibiotic properties. As our testing has not been concluded, there will be more data to be added.

6 Beaches and Bacteria Throughout Our Community

MEGAN JOHNSON, LEAH BRANTMEIER
Northeast Wisconsin Technical College

Today, we live in a world where antibiotics resistance is becoming the 'new normal'. As scientists begin to address this issue, they are starting to realize that the next decade could be completely different from the world we live in today. Throughout the course of 16 weeks, we have been growing bacteria in search of new antibiotics. Our overall hope for this project is to discover an unknown bacteria. The Tiny Earth organization has given students the chance to research local soil in hopes of finding new types of bacteria and present our results at a conference. We decided to investigate a popular hangout spot, and the beach was the perfect space. We collected soil samples from Sunset Beach and Duck Creek Quarry. Our hypothesis was that sunset beach would produce more antibiotics due to being on the bay rather than an enclosed body of water. Once we brought our samples back to the lab, we diluted our bacteria and streaked it on a plate. We then put them against both *Erwinia* and *S. epidermidis*. With this, we found that the Quarry soil had 13 positive producers while Sunset Beach only had 3. This showed that our hypothesis was correct and Sunset Beach had the higher amount of antibiotic-producing bacteria, but further testing must be done, and the final results are to be determined.

STUDENT POSTERS

7 What is Found in the Ground of Our Nursing Homes? Oddfellow and Edenbrook Nursing Homes Containing Antibiotic Producing Bacteria

KERISSA KARNOPP, CASSIDY LEE
Northeast Wisconsin Technical College

For a long time, there has been a problem that most people are unaware of in this world: antibiotic resistance. This is caused by people believing they don't feel the need to take the antibiotics they are prescribed for the length of their prescription, leading them to fall sick again, and the medication being useless. This has happened repeatedly, making simple things like an infection from a cut untreatable, leading to death. With the Tiny Earth project, we can try and find a solution at a low cost by having college students go out into their communities and collect samples of soil to find new antibiotics. We decided to collect samples from two different nursing homes: Oddfellow and Edenbrook. We predicted that the two homes would contain around the same amount of antibiotic-producing bacteria because they had the same social and natural environment. To find these results we diluted our soil using PBS and then plated them onto LB plates and put them off to the side for the week. We then transferred more bacteria onto another LB plate and a TSA plate and let it sit for another week, placing the LB plate in a dark environment to see if more bacteria would grow. After selecting 20 different colonies of bacteria, we placed each of them on a master plate and two test plates: *E. carotovora* and *S. epidermidis* and let them sit aside for a week checking after 24 hours for antibiotic activity. We found double producers on #20 for Edenbrook and #17 for Oddfellow. We then used an all-tester plate and a streak plate to test out our double producing antibiotic bacteria. After a week we found that both bacteria cleared on *S. epidermidis, E. carotovora, B. subtilis,* and *M. smegmatis*. Our results show that both nursing homes produced the same amount of antibiotic-producing bacteria which supports our hypothesis. This project and research are ongoing so there are more results to come.

8 Is Water Truly Healing?

SAMANTHA VANEREM, RACHEL DIEDRICH
Northeast Wisconsin Technical College

Since 1987, scientists have been struggling to find new antibiotics to fight back against antibiotic resistance. Antibiotic producing bacteria can be found in many different natural environments, such as soil near water. We chose to study the soil from a retention pond in Bellevue and the Fox River at Voyager Park in De Pere. These locations address the idea of finding new antibiotics because each body of water can contain different bacteria. We believed between the two soil samples that we would find an antibiotic producing organism. In the lab, we diluted and plated soil samples to isolate bacteria. We then tested for activity against relatives of the ESKAPE pathogens. We also tested the bacteria in other conditions on different types of agars to see if it affected the growth. After further testing against different strains of pathogens, each site displayed one clearing against a different pathogen; the river to *Mycobacterium smegmatis* and the pond to *Erwinia carotovora*. Since each bacteria appeared to only show antibiotic activity against a single pathogen, our hypothesis of finding an antibiotic from at least one soil sample was incorrect. Our research is still ongoing as we are waiting on genetic testing results to identify our bacteria.

9 The Effects of Climate Change on Antibiotic-Producing Soil Bacteria in Northeast Wisconsin

SONNY PAMONICUTT
College of Menominee Nation

There are many medicinal plants located throughout the Menominee reservation. Using two different plants, Plantain and Mullein, located on the College of Menominee Nation Phenology trail, soil samples were collected from each plant site, then tested. This study sought out to identify if climate affects the number of antibiotic producing bacteria found in the soil. This study replicated two previous students' study to identify a correlation to climate. Soil collected in late October from Plantain and Mullein were grown on Potato Dextrose Agar and Nutrient Agar. All samples were tested against safe ESKAPE pathogens using Tiny Earth Protocols. The study found positive correlations to antibiotic activity occurring during an El Niño year causing warmer weather patterns in North Eastern Wisconsin. This study may advance climate research and grant funded projects at the College of Menominee Nation. This study will also help reinforce the medicinal uses of these plants.

STUDENT POSTERS

10 Digging for Answers: How to Help Combat Antibiotic Resistance

KIM RUDNESS, KATIE GILKES
Northeast Wisconsin Technical College

Bacteria have been evolving with our misuse and overuse of antibiotics. They mutate to survive despite our best efforts to kill them. The Tiny Earth project was created for students and instructors to discover the world of antibiotic-producing microbes and help in the fight against our microscopic rivals. We decided to try two different locations near water sources with different soil textures. One sample came off a lake source in Neenah, WI, while the other was a sample in clay soil near a pond in De Pere, WI. Our hypothesis was that we would each find antibiotic-producing bacteria in each of our soil samples. We extracted bacteria from our two soil samples to see if we produced any antibiotic-producing bacteria by testing them against safe relatives of the ESKAPE pathogens. Then we used PCR and biochemical tests to ID our organisms. The sample from Neenah had two possible antibiotic-producing microbes compared to De Pere, which had one possible antibiotic-releasing microbe. The one from Neenah had a very interesting red/pink color to one of the possible microbes compared to the others that grew from the sample. Both of our microbes showed activity against the pathogen *Acinetobacter baylyi.* Further testing is in process to find the exact species of our microbes; however, our hypothesis that both samples would have antibiotic-producing bacteria was accurate. We are continuing our research and we are waiting for further results.

11 Identifying Antibiotic-Producing Soil Bacteria in the Omāēqnomenēw Maeqtekuahkihkiw

MARY HUNTINGTON
College of Menominee Nation

The Menominee Reservation is known for its vast, dense forest. Using the soil at the base of a Red Maple, located on the College of Menominee Nation phenology trail, soil samples were collected and tested. The soil sample was collected during late October from the base of the Red Maple tree. The study was conducted to identify soil bacteria that were producing antibiotic capabilities. The sample was grown on nutrient agar using Tiny Earth protocols. The sample was tested against a safe ESKAPE pathogen; *Bacillus subtilis*. This study found no antibiotic activity against the safe ESKAPE pathogen, however, this research can be added to further climate research and grant funded projects at the College of Menominee Nation.

12 What's Dirt Got to Do with It?

KENDRA RACETTE, JESSICA PRENTICE
Northeast Wisconsin Technical College

The world is always changing and bacteria is no exception. Old antibiotics aren't as effective as they once were due to bacteria adapting to survive after exposure to these medicines, and new antibiotics aren't standing the test of time. Nationwide many colleges have decided to implement a curriculum focused on solving this problem, the Tiny Earth Project. College students have been collecting local dirt samples to experiment with in the hopes of discovering bacteria that will produce a new antibiotic. We collected samples from a swamp and river basin. We predicted finding one antibiotic-producing microbe in the swamp and two antibiotic-producing microbes in the river basin. We used soil dilution to form isolated colonies to perform tests on. One such test was an all tester plate to test for antibiotic activity against relatives of the ESKAPE pathogens. The swamp produced a positive result against an ESKAPE pathogen relative, *B. subtilis*. The river bacteria showed no activity against the relatives of ESKAPE pathogens. This means the positive result from the swamp could produce an antibiotic. Our microbes are currently undergoing genetic and biochemical tests to be identified.

13 Tiny Earth Research

TAYLOR AUBRY
Northeast Wisconsin Technical College

Antibiotic resistance is a worldwide problem. New antibiotic-producing bacteria have not been discovered since the late 1980's. Tiny Earth is a project conducted at NWTC by students to help possibly discover a new antibiotic-producing bacteria from soil. Students gather soil samples from their chosen environments and conduct testing to identify a specific bacteria cultured. I predicted there would be at least one new bacteria discovered from this class's research. The soil collected was five and a half inches below the surface in the organic/surface horizon. The soil sample was then diluted 1:10,000 dilution to try to separate different colonies of bacteria to choose one of possible antibiotic-producing qualities. I selected a bacteria from my master plate I thought would be a new antibiotic-producing bacteria and did a pure culture on a new LB plate. Then I took a sample from the pure culture and tested it against nine other bacteria to see if my chosen bacteria showed antibiotic producing tendencies. The experiment showed one small zone of inhibition between the chosen bacteria and *Enterobacter aerogenes*, there were no zones of inhibition between the chosen bacteria and *Staphylococcus epidermidis*. The chosen bacteria colony morphology was bright orange in color, smooth, glistening, and convex; the edge was circular and entire.

14 The Impact Soil Has on New Antibiotic Research

ERIKA CASTILLO, YUGENTHINI THEIVENDIRAN, GAYATRI DHAKAL TIWARI
Northeast Wisconsin Technical College

As we know, the majority of antibiotics in use today are derived from soil bacteria. Which makes it perfect for us to find new potential antibiotics among the soil reservoir of microbes. We decided to join as a group and chose different areas to select our soil from. We picked soil near a restaurant, near train tracks, and near a fire department building. From the three places we went we thought topsoil from the train tracks might have the highest microbe content, as most bacteria can be found in topsoil rather than subsoil and subsoil from restaurant might have the least bacteria in it. Soil from train tracks could have the most microbe content since usually trains and people go through them at a constant rate. The fire department building may have been exposed to a variety of microbes which usually are carried by the fire trucks. Once soil was collected, we did serial dilutions to separate microbes from soil. We later tested our soil plates with *S. epidermidis* and *Erwinia* to see whether our microbes produced antibiotics to kill those pathogens. We found that soil from train tracks had 3 bacteria that produced antibiotics. Soil from the restaurant only had two and soil from the fire department gave 9 unique bacteria which produced antibiotics. We found that we were wrong with our prediction and the soil that produced more microbes was soil from the fire department. This could be because of the type of soil and the area it came from. Our microbes are under ongoing investigation.

15 Discovering the Antibiotics That Live in Our Backyards

KALISSA KELSEY, EMILY CALAWAY, JONATHAN ANDERSON
Northeast Wisconsin Technical College

The Tiny Earth research project is all about finding what lies beneath the soil. Due to the lack of knowledge provided to patients about antibiotic treatments, we have been forced to halt the production of more antibiotics for years. With the discovery of new antibiotics, we can inform patients of the proper use, and avoid resistance! To begin the research, we collected soil from our very own backyards. For each collection of soil from different sites, we predicted we would find a minimum of one antibiotic producer from one of the three sites. When we tested the bacteria collected from the soil against ESKAPE pathogens, we hypothesized that we would see a reaction happening between the ESKAPE pathogens and bacteria. Isolating colonies within our group we were able to select ideal growing conditions for our bacteria. With the growth of more bacteria, we performed a test to check what colonies preferred growing on *S.epidermidis* which is a gram-positive bacteria, and a gram-negative bacteria called *Erwinia*. Results from these tests that were conducted showed more activity for all three of our chosen bacteria against *S.epidermidis* which was the gram-positive bacteria that we tested against. When we tested our chosen bacteria against the ESKAPE pathogens, we found that each of our chosen bacteria preferred to kill bacteria that were gram-positive, specifically *B.subtilis*. We found that in the end, the locations we used for our soil collection all had ideal bacterial genetics that we used to further our research to find an antibiotic producer.

16 Identifying Antibiotic-Producing Soil Bacteria at Different Soil Depths

NICHOLE VERSTOPPEN, JESSICA WILLIAMS
College of Menominee Nation

There are many medicinal plants located throughout the Menominee reservation. Using two different plants, Plantain and Mullein, located on the College of Menominee Nation Phenology trail, soil samples were collected from each plant site at soil depths of 8 cm and 13 cm, then tested. This study sought to identify if soil depth has an effect on the amount of antibiotic-producing bacteria found in the soil. Soil collected in late October from Plantain and Mullein were grown on Potato Dextrose Agar and Nutrient Agar. All samples were tested against safe ESKAPE pathogens using Tiny Earth Protocols. The study found 38% of the microbes from the 8cm depth that were tested were positive for antibiotic production and 67% of the microbes from the 13 cm depth that were tested were positive for antibiotic production. This study may advance climate research and grant funded projects at the College of Menominee Nation. This study will also help reinforce the medicinal uses of these plants.

17 Soil from the Northwoods

KELLY WHITE
Northeast Wisconsin Technical College

For years, people have gotten sick repeatedly, and antibiotics have started to lose their effectiveness. Now scientists are faced with the problem of no new antibiotics being discovered in recent years. My Microbiology class was tasked with an exciting chance to become part of scientific history by taking part to help find new antibiotics to help treat our population. My hypothesis was that I would find five antibiotic-producing microbes. I set my goal high since I chose a deep location for my soil sample compared to others in my class, 27 inches from ground level. First, I diluted my soil sample, then I streak plated it. From there, I cultured the soil bacteria in a variety of growth conditions such as using a Luria broth (LB) agar plate incubated at 37 degrees Celsius and a Tryptic Soy Agar (TSA) Plate at room temperature. I then took my soil sample and tested against *Erwinia carotovora* and *Staphylococcus epidermidis*. There were two zones of inhibition that were double producers. Next, using the sterile loop technique and four phase streaking method, I used my all-tester plate and compared it against nine different ESKAPE pathogens. I had one more additional zone of inhibition; it was with *Escherichia coli*. My hypothesis was not accurate. I hypothesized that I would find five antibiotic-producing microbes and I found three.

18 Antibiotics in our Quarries and Gardens

TIXARELI ARANDA, SAVANNAH HOUSE
Northeast Wisconsin Technical College

Over the last years as we have been advancing, so have all microbes that live, and want to live within us. They have evolved to avoid being killed off by antibiotics and it has become a problem for all of us. That is why my peers and I became part of the Tiny Earth project. We chose bacteria from random places all around the community to see if we were capable of discovering new antibiotics from the soil around us. My partner chose her soil from the Redgranite Quarry, and I chose soil that was being used to garden apple trees. We predicted that we would find an antibiotic-producing microbe due to our samples coming from places that produce and are surrounded by life. From our own samples, we chose different bacteria to test. My partner chose the microbe that showed activity against *Staphylococcus epidermidis* while I chose bacteria that worked against *Enterobacter aerogenes*. From there, we were able to begin our testing. The first test was a PCR test which lets us know whether our bacteria had the antibiotic-producing gene. The Redgranite Quarry bacteria showed this gene while the garden soil didn't. We will continue to run tests on these bacteria and find either an antibody-producing microbe or a completely unknown microbe.

19 Old McDonald had an Antibiotic

ANNA MEYER, EMILY TYEPTANAR
Northeast Wisconsin Technical College

Since 2020, we have been faced with a global Pandemic (Covid), but little did we know we would be facing a bigger global problem. Scientists have been hard at work with trying to discover new antibiotics based on alarming statistics of antibiotic resistance. Through the Tiny Earth Project, we are trying to find new antibiotics in our community that could be present in your own backyard. In our research, we wanted to compare soil samples between a beef farm located in Waupaca, WI and a hobby farm located in Newton, WI. We believed that the beef farm would have a higher chance of making antibiotic-producing microbes due to the continuous use of antibiotics of that one specific species, whereas the hobby farm has over six different species that do not use antibiotics. We collected dirt from those sites, and then in class we diluted the soil bacteria to make isolated colonies using the LB plates. Looking at these plates, we chose the best isolated colony growth. We then compared that bacteria to eight relative ESKAPE pathogens, and discovered that the hobby farm sample had activity against two pathogens, *E. coli* and *A. baylyi* as compared to the beef farm, which had no producers. The experiment is ongoing, more results are to come.

STUDENT POSTERS

20 ESKAPE the Bay

RACHEL KLOSIEWSKI, ALEXIS BOMSKI
Northeast Wisconsin Technical College

Antibiotics have been a notable finding in science and healthcare over the past decades, due to their ability to treat bacterial infections. The consequences of their common use or misuse among our population, however, has caused some bacteria to mutate and become antibiotic-resistant. These bacteria strains are classified as the ESKAPE pathogens, as they can be very dangerous due to their ability to survive antibiotics. The goal of the Tiny Earth Project is to explore different microorganisms in the soil and test if they produce antibiotics by isolating them and observing their interactions with commonly known bacteria. We hypothesize that there will be three antibiotic producers shown in our project, whether that be already known antibiotics, or unknown antibiotics. There were soil samples taken from Communiversity Park in Green Bay and a backyard near farmland. The sample from near the bay was chosen because the park has many different details near it that could impact its microbial composition such as water, sand, trees, plants, and moss. The soil near the farm was chosen because we hypothesize it has many different microbes needed for crops to grow.The methods of this experiment were to collect a sample of soil, dilute and grow bacteria from it and test them for activity against relatives of the ESKAPE pathogens. Clearing, or empty spaces between streaks of bacteria indicate that the bacteria could not grow due to antibiotic activity from the bacteria in our soil samples.The clearing on the soil sample plate from near the Bay was on the sections with *S. epidermidis, E. carotovora, P. putida*, and *B. subtilis*. This is evidence that the soil from Communiversity Park has antibiotic producing bacteria against those ESKAPE pathogens. The sample taken from near the farmland had antibiotic activity around *S. epidermidis*. Our hypothesis was proven correct, as there were at least three sections that showed antibiotic activity. These results mean that there are many antibiotic-producing bacteria in our environment that could potentially kill pathogenic bacteria.

21 What Is Growing Under Your Cows, Goats and Deer?

ABIGAIL WETZEL, KAITLYN KASTENSON, STEPHANIE REZNICHEK
Northeast Wisconsin Technical College

Alexander Flemming was a Microbiologist who discovered the common antibiotic, Penicillin. This would come to be the first known antibiotic discovery. Since this discovery in 1928, bacteria has been mutating and becoming resistant to the known antibiotics. As a group, we collected soil samples from three different animal occupied areas in hopes to discover an unknown, non harmful to humans, antibiotic producing microbes. We predicted that we may find a few antibiotic microbes to work with. The first sample was collected near a cattle pasture, the next, a goat pasture, lastly, a nature reserve where deer and other animals graze. To discover any antibiotic-producing microbes we diluted our sample, grew many colonies of one specific bacteria, We tested our isolated bacteria against relatives of the ESKAPE pathogens, then performed genetic testing to determine what microbes we identified and ran biochemical tests to determine metabolic activity. We found some promising results as there were clearing within our tests suggesting antibiotic activity. Our research is still ongoing so we are awaiting our further genetic and biochemical tests.

22 Antibiotic Producers Collected from Organic and Non-Organic Farms

AMANDA RIDDERBUSH, ANN BIRNSCHEIN
Northeast Wisconsin Technical College

The “post-antibiotic era” refers to when antibiotics, the drugs we use to treat bacterial infections, become significantly less effective or even useless due to the widespread development of antibiotic resistance. Antibiotic resistance occurs when bacteria evolve and adapt to survive the effects of antibiotics, rendering these drugs less or completely ineffective. Due to this crisis, The Tiny Earth Organization has presented opportunities for our class to help research new antibiotics in the soil around our area. We chose samples from two areas in Door County to investigate the antibiotics in non-organic and organic farming. Could spraying fields against harmful bacteria kill the good antibiotics as well? One sample was taken from an organic potato field and former apple orchard, and the other was taken from a non-organic corn field to compare which would possess more antibiotics. We hypothesized that the organic farm would yield more antibiotic-producing bacteria. After collecting our soil, we diluted the soil samples to obtain isolated colonies. We then tested the diluted colonies against *Erwinia* and *S. epidermidis*. The organic soil had seven samples that showed bacterial clearing including one that was a double producer, and the non-organic only had three. We selected one sample from each isolate to test against the remaining ESKAPE pathogens. The next step we took was to perform a colony PCR test followed by gel electrophoresis of the PCR products. Both of our samples tested positive on the gel electrophoresis. Our tests are still ongoing as we plan to do Gram staining, biochemical experiments, and DNA sequencing to help identify our isolates.

23 Searching for Bacteria with Antimicrobial Properties in Wildlife Soil

AUBREY WATZKA, CASSIDY BOESEN, NICOLE LUBENSKE
Northeast Wisconsin Technical College

The discovery of antibiotic-producing bacteria has changed the face of the medical world, as antibiotics can be used to treat bacterial infections. However, in today's society the extreme overuse of antibiotics has led to antibiotic resistance. The Tiny Earth research project allows students to test soils in their neighborhoods for new antibiotic-producing microbes which can be used to overcome antibiotic resistance. To do this, we obtained soil samples from three different rural locations in the greater Green Bay area. Our sample locations included old swine pen grounds, wetland soil with frequent wildlife traffic, and a manure pile from a horse stable. Our hypothesis was that the sample obtained from the wetland location would contain the most microbes with antimicrobial properties than the other two locations, as the environment is exposed to a complex multi species environment. To test our soil, we diluted and plated our three soil samples. Next, we tested our isolated bacterial colonies against *S. epidermidis* and *Erwinia* to see if our bacteria killed either of them. After looking for zones of clearing, we each chose one bacterial colony from our samples to test against relatives of the ESKAPE pathogens. Our results showed that the three colonies we chose had no antimicrobial properties. Therefore, our hypothesis was not accurate as none of our samples had any antimicrobial properties despite the varying exposure to different species of wildlife. Our research on the soil from our three sample locations is still ongoing, and further results remain to be determined.

24 Running Out of Antibiotics and Time

NICOLE RUSSELL
Northeast Wisconsin Technical College

There is a huge problem for every person on this planet and it is becoming a greater problem every year. Bacteria are increasingly becoming more resistant to antibiotics and thousands of people are dying every year; with this number continuously increasing. Tiny Earth has brought many colleges together to have students work on finding antibiotic-producing bacteria that have not yet been found. A new antibiotic-producing bacteria can help fight the many resistant diseases that are killing people. This is done by collecting soil samples from anywhere you want and testing them in the lab at school. My thought was that I would have at least a couple antibiotic producers. I diluted my soil sample down and transferred a sample to a Luria broth plate for colonies to grow. Although in my initial test I didn't have a lot of colonies growing, I did have success by using a Luria broth plate at 37 degrees Celsius and a Potato Dextrose Agar plate at room temperature. I selected twenty microbe colonies and inoculated them on plates of *Staphylococcus epidermidis* and *Erwinia carotovora* to see which ones would produce antibiotic tendencies against them. I ended up with four microbes that were possible antibiotic producers. I chose one of those microbes to test against the ESKAPE pathogens and created a streak plate for future research. My hypothesis was correct, and my microbe came back with two possibilities of antibiotic producers against *Enterobacter aerogenes* and *Bacillus subtilis*.

25 What's Under Our Feet

NAKEISHA HOLDER, MELODY PAYNE
Northeast Wisconsin Technical College

Disease-causing bacteria have become resistant to the many antibiotics over the years. With more antibiotics not being suitable treatments anymore, the discovery of more antibiotics is needed for the future of human life, this is what the Tiny Earth organization alongside colleges have been researching. We took samples of soil in separate locations of Green Bay: The NWTC Butterfly Garden, and the parking lot of Tower Clock Eye Center. Our hypothesis was that we would find at least one to two microbes that have antibiotic agents based on them being in deep soil with many organisms around the soil. We did soil dilutions, and smeared culture plates to grow bacteria. We then tested the bacteria against *S.epidermidis* and *Erwina* to see if they pro]duced antibiotic activity. The NWTC Butterfly Garden had three potential microbes releasing antibiotics, the parking lot of Tower Clock Eye Center had one potential microbe releasing antibiotics. We then tested for antibiotic activity against the relatives of ESKAPE pathogens. The NWTC soil had one microbe test positive against

E.carotovora, and Tower Clock Eye Center did not have any microbes test positive against any of the pathogens. Our hypothesis was correct since we did find at least one antibiotic-producing microbe in our soil. Our research is continuing with microbes and further results will be presented in the future.

26 Conducting a Quest for Antibiotics Within Soil

HAILEY ZUELKE, MYA MARTIN, GAVIN SRNKA
Northeast Wisconsin Technical College

One of the greatest threats to current global health, food security, and development is antibiotic resistance. When bacteria adapt to the use of antibiotics, antibiotic resistance develops. To begin we collected our soil from three diverse areas: farmlands, ditches, and areas close to rivers. Because those areas have a high level of agricultural influence, all three of us predicted to find at least one antibiotic-producing microbe in our soil samples.We initially began with soil dilution, incorporated new conditions and gathered unique colonies. We tested the bacteria against *S. epidermidis* and *Erwinia*. Further testing was done on the relatives of the ESKAPE pathogens. The soil from the farmlands first showed clearing against *S. epidermidis*. The soil from the ditch initially showed clearing against *Erwinia*. The soil near rivers was found to have no clearing against anything. Those results prove our hypothesis wrong. Currently, our tests are still ongoing.

27 Yooper Dirt

KAYLEE CAVIS, MORGAN CAPPAERT
Northeast Wisconsin Technical College

Today, there is a problem that the world is facing, and that just so happens to affect every single one of us, including your future children, grandchildren and so on. We are running out of antibiotics used to fight bacterial infections. That is where the Tiny Earth project comes in. My partner and I go to NWTC, and we have had the opportunity to help stop this crisis that we are facing and attempt to find a new antibiotic! For this project we decided to take dirt from Upper Michigan. What made us decide to collect our soil samples here is that we are both from Yooper country. We collected soil samples from the shores of Lake Superior and Torch Lake. We chose to collect soil by Torch Lake because we thought that the dirt by the abandoned Quincey Mine in Hancock, Michigan, may have mutated and started growing antibiotic-producing bacteria. We chose to collect the soil along Lake Superior because it goes through so many different climate changes and has plenty of plants and trees. Because of the more visible life from the soil, we hypothesized that Lake Superior would produce more antibiotic resistance. So far, our experiment showed four antibiotic-producing microbes: two of them from Lake Superior and the other two from Torch Lake. Our experiment is still ongoing, but we hope to have answers for you all come time for the presentation!

28 Antibiotic Producing Bacteria Obtained From Cow and Horse Manure

GRACIE LILLGE, ALYSSA WESELY
Northeast Wisconsin Technical College

The discovery and importance of antibiotic treatment has saved many lives. Although over time bacteria have learned to develop a resistance to antibiotics. Mutations can occur due to overuse or misuse. The antibiotic-producing bacteria living around us have allowed us to study and research antimicrobial production within soil. Our research project aims to compare two soil samples; composted cow manure and composted horse manure. Both samples have been used as garden soil and we thought this comparison would be interesting because it is manure from two different animals. We hypothesized that the soil with the cow manure would have the most antibiotic-producing bacteria. We made this hypothesis because we know that horses have relatively poor digestive systems compared to cows. We diluted, streaked, and plated our soil samples, and the microbes that were found in our samples were then plated against *S. epidermidis* and *Erwinia* to test for antibacterial producers. We each picked one antibacterial producer and plated them again to test against ESKAPE pathogens. Our hypothesis was proven correct, showing that the soil sample with cow manure contains more antibiotic-producing bacteria than the soil sample with horse manure. Considering our research is still in progress, more results will be collected and shared.

29 Tiny Earth

LEXIE BERGNER
Northeast Wisconsin Technical College

For many decades now, scientists have been struggling with the problem of antibiotic-resistant pathogens and the inability to find “working” antibiotics. These struggles have led to an increase in mortality around the world. The Tiny Earth organization has allowed students to research soil in our communities to find microbes that release new antibiotics and then present our findings at a conference. I have decided to research the soil at my house by my firewood storage. I have decided to do this because my dogs love to play over there. My hypothesis was that the soil at my house would contain two antibiotic-emitting microbes that would be dual producers. I diluted and plated my soil from the sample of soil from my house on a plate using the streak plate method. The microbes we then found were inoculated and screened against the bacteria *Staphylococcus epidermidis* and *Erwinia carotovora* to see which microbes would produce antibiotics against the different bacteria. My sample had four producers and none of the microbes were dual producers. I then chose one of my antibiotic producers and screened them against the ESKAPE pathogens. My results showed that the soil from my house contained four antibiotic-producing bacteria. My hypothesis was not accurate because although I did have four antibiotic-emitting bacteria found in my soil, none of them were dual producers.

30 Backyard Soil Leading to Possible Antibiotic

KYRA GERUE
Northeast Wisconsin Technical College

The issue that Tiny Earth is assisting in is that no new antibiotics have been discovered in nature since late 1980's. This has resulted in the overuse of available antibiotics, which has now led to antibiotic resistance. This means the bacteria are mutating and multiplying faster and becoming harder to fight. Thanks to the Tiny Earth project, students can take soil samples from our choice of location and conduct tests with them in hopes someone can find a microbe that will produce a new antibiotic. I obtained my sample from the backyard next to the raised flower bed where there are some Hosta plants nearby. I believe my soil will have a few antibiotic-producing microbes, but unfortunately, I don't think there will be a novel antibiotic. We performed several tests and procedures starting with serial dilution of the soil sample. I then cultured soil bacteria in a variety of conditions to see if there was an impact on growth. The microbes were then tested against *S.epidermidis* and *E. carotovora* to assess for antibiotic activity. I had two dual-producers. Then we screened against the ESKAPE pathogens which resulted in 2 new zones of inhibition.

31 What is Moo-ving Beneath the Surface?

NADIA QUICK, ABBY SAUTER
Northeast Wisconsin Technical College

For many years, doctors and physicians have been struggling to treat antibiotic-resistant pathogens that are affecting people all around the world. In response, scientists have been looking for ways to create new antibiotics to treat these infections. The Tiny Earth project has outsourced their research for college students to examine the soil in our communities in hopes of finding microbes that can produce antibiotics. We collected soil samples from a farm field and next to a calf hut on a different farm. Our hypothesis was that we would discover at least one antibiotic-producing microbe. We thought that the soil from these areas would have higher numbers of antibiotic-producing microbes due to the presence of live animals and fertilizers. We diluted the samples and used the streak plate technique to grow the microbes that were present. The microbes were then inoculated and grown with the bacteria *S. epidermidis* and *Erwinia* to see if any of the microbes produced antibiotic activity against the bacteria. Neither sample contained antibiotic-producing microbes, so we chose a colony that stood out from the others. This was then tested against relatives of the ESKAPE pathogens. Our results once again showed that we did not have any antibiotic-producing microbes. Our hypothesis was incorrect, since there were no antibiotic-producing microbes present in either sample. However, the experiment is still ongoing, and we will continue to test the microbes for identification purposes.

32 Can 3 Different Environments Give One Type of Antibiotic?

MARINA MUNSCH, EMILY JENKINS, NIRAMOL YODSIRISUK
Northeast Wisconsin Technical College

For the past 36 years, scientists have struggled to discover new antibiotic treatments due to the overuse and misuse in healthcare. When antibiotics are misused, it allows bacteria to become resistant. The Tiny Earth Project is an opportunity for students to discover new bacteria that could be later used as new antibiotic treatment. Our group decided on testing soils in three different types of gardens: a private home-garden, NWTC's public botanical garden, and a local hiking trail. These spots were chosen to see how a difference in foot-traffic, landscaping practices, and environmental factors would affect the types of bacteria that were found. It was predicted that the two public locations would have two antibiotic discoveries each. The thought of chemical use and other landscaping practices in the home-garden affecting bacterial growth led us to hypothesize that only one bacterium would be discovered in that location. We used soil dilution to isolate bacterial colonies from each of the three sites and tested them against known pathogens. The sample from NWTC's botanical garden had six antibiotic producers, including two double producers. The public trail had three antibiotic producers and the sample from the private backyard garden had five antibiotic producers. The results showed our hypothesis was incorrect however there are more ongoing experiments to determine the identification of these bacteria.

33 Antibiotic Producers Found in Peshtigo, Wisconsin

MYLEE DAVIS
Northeast Wisconsin Technical College

Over the past few years, antibiotic resistance has become a serious issue. People have lost their lives due to antibiotic-resistant pathogens. Scientists are having a very difficult time discovering new ones. With the Tiny Earth research organization, students have been provided an opportunity to make history. Soil samples in communities are obtained with hopes of finding new microbes to help find new naturally occurring antibiotics. This research gives students opportunities to show off their countless hours of work in the lab. I obtained my soil sample in my backyard. I live in Peshtigo, Wisconsin and was not very confident in what I might find. I hypothesized that I might find two microbes emitting antibiotic properties due to the fire that happened in 1871. I first performed a serial dilution on my soil sample which resulted in one hundred colonies, eight of which were unique. I also tried to recreate the environment in which I obtained my soil sample, my LB dark box was most successful while doing this. I then went on to test my bacteria against *Staphylococcus epidermidis* and *Erwinia carotovora*, which had two zones of antibiotic activity and zero double producers. Lastly, I tested my selected bacteria against nine different types of bacteria which included ESKAPE pathogens. I found two new zones of inhibition which were on *Escherichia coli* and *Pseudomonas putida*.

34 Tiny Earth Project; Will an 1800's Cemetery House Unknown Bacteria?

REBECCA SOBIECK
Northeast Wisconsin Technical College

Antibiotic resistance is a growing problem in our population caused by multiple factors. Scientists are trying to research new microbes that could bring advancements in the journey of antibiotic discovery. My hypothesis is that the 1800's cemetery will house at least 1 antibiotic-producing microbe in its soil from the decomposing bodies lying in the graves. The goal is to identify a new microbe in which the scientific community could use against the antibiotic-resistant crisis. In order to accomplish this, soil was collected and taken to the lab, in which it went through a process of purifying and cleaning the sample. Then sent out for genetic testing to identify the makeup of the sample. Once sequencing results came in, my sample results came back as contaminated. Unfortunately not identifying the exact microbe. In conclusion, I was incorrect in my hypothesis because I found three antibiotic-producing microbes in my soil sample instead of my predicted amount being one.

35 Antibiotic Producers in Brookside and Howard, Wisconsin

KLAIRE O'LEARY, JAIDAN CONLON
Northeast Wisconsin Technical College

Antibiotic-resistant microorganisms are becoming more prevalent in many healthcare settings around the world. Healthcare workers are finding that the harm caused to patients who have contracted these antibiotic-resistant microorganisms is increasing rapidly, and we are currently struggling to find ways to treat these infections. The end goal of collecting and testing the soil is to find if we have any antibiotic-producing microorganisms in our samples. We collected soil from two very different locations, one being a farm field in Brookside and the other being a sports complex in Howard. Our hypothesis was that the farm field would be more likely to have antibiotic producers due to the amount of nutrients that are supplied to and grown in the soil. We began by diluting our samples and plating bacteria, followed by screening the microbes found against *Erwinia* and *S. epidermidis*. The soil collected from the sports park had three possible antibiotic-releasing microorganisms while the farm field only had one. We each chose one of our antibiotic producers and screened them against relatives of the ESKAPE pathogens. Our results showed that both soil samples had activity against *E. coli, E. carotovora, A. baylyi*, and *B. subtilis*. The sample from the farm field also showed activity against *E. aerogenes*. We had found that our hypothesis was correct, the farm field did have more antibiotic producers than the sports park. The experiment is ongoing with genetic and biochemical testing to identify the organisms.

36 To Swim or Not To Swim?

CASSIE DUPREY, DESTINY TORRES
Northeast Wisconsin Technical College

The purpose of our Tiny Earth project is to find out if we have antibiotic-producing bacteria at a public, local, sandy, swimming area. Our aim is to find out if the Duck Creek Quarry Park Beach in Howard, Wisconsin harbors more antibiotic-producing bacteria on one side than the other. We obtained two different samples from two different areas of the beach, the adult swimming area versus the child friendly swimming area. We hypothesized that the child friendly site would contain more diverse bacteria with at least one with antibiotic property as compared to the adult beach. We believe this because normally children are more likely to harbor bacteria than adults, given the nature of children. Through soil collection, soil dilution, and different types of media for growth incubated at different temperatures, bacteria grew at different rates. We also tested our bacteria for hemolysis, starch hydrolysis, and catalase tests. We compared our bacteria to ESKAPE pathogens to see if there would be any antibiotic reactions. Our bacteria did not have any activity against any of the ESKAPE pathogens from the children's beach, but the adult beach had antibiotic activity against *E. carotovora*. To further identify the pathogens, we Gram stained our bacteria which resulted in both bacteria as Gram positive. Both were further identified under microscopy; the children's beach produced streptococcus whereas the adult beach produced streptobacillus.

37 The Quest for Novel Antibiotics

ADAM TOUCHINSKI, MAKENZIE MICHAEL
Northeast Wisconsin Technical College

Antibiotic resistance within the medical field has become a serious issue when treating specific types of bacteria. The last novel antibiotic to hit the market was in 1987 and the number of uncontrollable infections continues to rise. It was our quest to seek out and identify new antibiotics within our local communities. We each selected a local park near our homes and collected soil samples. One sample was collected from High Cliff State Park predicting at least two antibiotic-producing bacteria would be found, and another from Wiese Park predicting at least one. We added our samples to a buffer, diluted them, then streak plated and cultured the dilutions. Then created a master plate and two tester plates, one inoculated with *Staphylococcus epidermidis* the other with *Erwinia*. After 24 hours we found that the High Cliff sample had two antibiotic-producing colonies and the Weise sample contained three. We each chose one antibiotic-producing colony to test against relatives of eight ESKAPE pathogens. It was found that the Weise sample showed activity against *Bacillus subtilis*. The High Cliff sample showed activity against *Mycobacterium smegmatis*. Our initial hypothesis was partially correct as one of our samples indeed had one antibiotic-producing bacteria. Research is still ongoing as to the identity of the microbes and their individual metabolisms.

38 Man's Best Friend, Now Even Better

OLIVIA SEERING, KRISTEN HUNTER
Northeast Wisconsin Technical College

Alexander Fleming completely changed the game with his discovery of penicillin against bacterial infections in 1928. Since then, we have taken penicillin and other found antibiotics for granted and now are at an all-time low. The last antibiotic introduced and successfully implemented in health science was in 1987. It is our job now to discover new antibiotic activity throughout nature. We, as a group, decided to investigate dog parks as man's best friend loves to run in the mud and bring that bacteria home. As a very popular spot for humans and their fur-children, we wanted to see if there was antibiotic activity with the bacteria around there. We began by collecting soil samples from two different dog parks in the Green Bay area, specifically the Brown County Dog Park and the Bellevue Dog Park. We decided on these two dog parks to determine if a wooded, pre-existing area (Brown County) would have more antibiotic-producing bacteria rather than a newly constructed one (Bellevue). To our surprise, we both only found one antibiotic-producing bacteria from each dog park. After this, we diluted the soil to get isolated colonies. We then looked at the activity between *Erwinia* and *Staphylococcus epidermidis*. From that, we tested the antibiotic activity between ESKAPE pathogens. As we continue studying these bacteria, our next step is to identify the specific bacteria we acquired from each dog park to further understand it.

39 Antibiotics Lurking in the Dark

RILEY AUTORE
Northeast Wisconsin Technical College

Antimicrobial resistance is a huge emerging public health problem. Since 1987, there has been a "discovery void" and no new classes of antibiotics have been discovered. Since there are more than 2.8 million antimicrobial-resistant infections in the U.S. each year, it is important that we make our best attempt to find new classes of antibiotics. The Tiny Earth organization gives me the chance to research the soil in my front yard in hopes to find new antibiotic releasing microbes. It is my hypothesis that my soil will show antimicrobial properties, but it will not be a new microbe, and it will grow best in a dark room since the sample was taken from under a tarp. I diluted and plated my soil using a serial dilution technique. The microbes found were inoculated and screened against the bacteria *Staphylococcus epidermidis* and *Erwinia carotovora* to see which microbes were sensitive to antibiotics produced by my soil bacteria. My *Staphylococcus epidermidis* plate produced three zones of inhibition and my *Erwinia carotovora* produced two zones of inhibition, both of which were double producers. We then tested our samples against nine other bacteria to search for any additional zones of inhibition, which I did not have.

40 Country Side to the City

QUINCI TAPPA, JONAH PIGEON, SARAH LEITERMAN
Northeast Wisconsin Technical College

The overuse of the antibiotics we have has led to increased drug resistance in bacteria. Antibiotic soaps, creams, and over-prescribing antibiotics has led to what is known as the antibiotic crisis. The Tiny Earth Project gives college students the opportunity to be the ones to find the next antibiotic and to begin solving this crisis. Students took soil samples from local areas, City Park in Oconto, WI, Hornstead Dairy Farm in Brillion, WI, and St. Norbert Abbey, to isolate and test bacteria found in the soil, hoping to find one that produces a chemical that repels other organisms. City Park was hypothesized to have two antibiotic-producing organisms, Hornstead Dairy Farm hypothesized to have three, and St. Norbert Abbey hypothesized to have one antibiotic-producing organism. From this, the soil samples were diluted with PBS allowing us to plate and test the bacterial growth of our soil on different agars and at different temperatures. Obtaining these results from different types of agars, students selectively chose twenty isolated colonies to continue testing on. Testing continued so students were able to determine if the selected twenty colonies had any antibiotic properties. Students were then able to choose one bacterium to focus upon. The chosen bacteria either showed an antibiotic property or had other effects on surrounding pathogens of the production of antibiotic was not clear. Amid this point students furthered their testing on the chosen bacteria using relatives of the ESKAPE pathogens. If a zone of clearing appeared, it meant there may have been a preference by the antibiotics produced from the bacteria. Furthermore, students used gel electrophoresis and polymerase chain reaction test to help determine the DNA and exact species of their chosen bacteria. With the experiment ongoing, students will have more results to come.

41 Medicine's Best Friend

ALEXIS STEPHENS
Northeast Wisconsin Technical College

Antibiotic discovery first took place in 1928 – less than a century ago. A discovery that has changed medicine and saved the lives of millions of people. Unfortunately, pathogens have found a way to mutate and are now becoming antibiotic-resistant. The Tiny Earth project has made it possible for students to gather soil from their communities, in hopes to encounter a novel microbe that possesses antibiotics. I have gathered two different soil samples, both located within the Brown County Park Pet Exercise Area. The fist sample was collected on a well-used path towards the entrance of the dog park. The second sample was collected off to the side in a grassy, not so tracked area. My hypothesis was that more microbes with antibiotic properties would be found in the second, off-path sample than the first on-path sample due to the area not being as disturbed, allowing more microbial growth. Both samples were diluted and streaked onto separate plates with different conditions. The different microbes found were collected and screened against *Erwinia* and *S. epidermidis* to determine if any produced antibiotics. The on-path sample showed positivity for six different microbes, with one dual producer. The off-path sample showed positivity for only three different microbes. I then chose one positive microbe per sample to test against 8 different ESKAPE pathogens. The results have shown that my hypothesis was incorrect, the on-path sample produced more microbes with antibiotic properties than the off-path. My research is still ongoing; therefore, more results are to be determined.

42 Searching Farms for Antibiotic Producers

RILEY NELLIS, KENIA POLOMIS
Northeast Wisconsin Technical College

Antibiotic resistance is becoming a larger problem due to them being misused, which has been occurring since they were discovered. The issues with misusing an antibiotic start with not finishing the full course of treatment or taking too many at once which leads to the bacteria building a resistance. Taking antibiotics when you have a viral infection instead of bacterial infections is another popular mistake that occurs every day. For our project we took soil samples from a farm in upper Door County and a farm in Kaukauna, to research in hope of finding new antibiotics. We are presenting these soil sample findings because we found it interesting to compare the different bacteria that grow on farms. We both hypothesized that we will find at least one bacteria that will have antibiotic-producing microbes. One of the first methods we performed was soil dilution. We did this to reduce the concentration of the original soil sample to levels that are low enough for single colonies to be grown on media plates. We were testing our pathogen against organisms related to the ESKAPE pathogens, which are highly virulent and antibiotic-resistant pathogens. At the Door County farm, it was found that while the bacteria had no clearing or activity, it did have a bright pink color which was extremely intriguing. At the Kaukauna farm, it was also found that there was no activity or additional clearing. Through our research thus far, our hypothesis was not correct. However, this is an ongoing experiment and results may change.

43 Soil Samples Collected from Brown, Oconto and Waupaca County

ALAYNA KUROWSKI, MACKENZIE FAHLER, ASHLEY RADLOFF
Northeast Wisconsin Technical College

In 1928 Penicillin was discovered by Alexander Fleming, just short of a hundred years ago. Within this time, we have discovered several others but what happens when bacteria become resistant? Antibiotic resistance has been a problem for decades now. This led Scientists to produce the Tiny Earth Program to test different soil. The point of testing soil is in the hope of discovering new microbes that produce antibiotics to help resolve the issue of antibiotic resistance. In our research, we took soil samples from three different counties in hopes for a bigger variety of antibiotic-producing microbes. The three counties are Brown County, Oconto County, and lastly Waupaca County. Our hypothesis was that the soil from Waupaca County would have less producers than the soil from Oconto County and Brown County. We believe this because the soil from Waupaca County was an old dumping ground for sewage back in the 1950's. Oconto County's soil sample came from a wet environment near the Oconto River at Copper Culture Park. The Brown County's soil sample came from an accessible area in a backyard. The sample from Oconto County was very wet and sandy while the other sample from Waupaca came from an area that is covered in mossy grass with a mixture of sand, low level of clay and a bit of moisture type of environment. The sample from Brown County was very dry. We began testing our samples first by diluting the soil and plated our bacteria. We furthered our research by testing activity against multiple different bacteria and pathogens including the relatives of the ESKAPE pathogens. Our results concluded that all the samples had antibiotic-producing microbes, especially the soil from Waupaca and Brown County. Waupaca County showed activity against *Bacillus subtilis* and *S. epidermidis* organisms. The soil from Brown County showed activity against *S. epidermidis* and *Erwinia*. The soil from Oconto County had activity against *S. epidermidis* and *B. sublilis* which are two of the relatives of the ESKAPE pathogen. This resulted in our hypothesis not being accurate in that the soil from Waupaca County and Brown County produced more antibiotic-producing microbes than the soil from Oconto County. Our research is still ongoing with further testing in hopes of discovering a new antibiotic to help with this ongoing issue.

44 Tiny Earth

AMBER HENNINGER, HANNAH DEKKER
Northeast Wisconsin Technical College

The Tiny Earth project aims to address the growing issue of antibiotic resistance by engaging students and communities in the discovery of new, naturally occurring antibiotics from the soil. The project encourages participants to explore and research microorganisms found in local soil samples, with the goal of discovering potential new sources of antibiotics. This initiative not only promotes scientific discovery, but also seeks to contribute to the development of novel antibiotic treatments to combat the increasing problem of antibiotic-resistant pathogens. We predicted that the soil at Grafton Family Dental will have more antibiotic-producing bacteria because there is a variety of things in the soil like rocks and mulch. To find bacteria that can be possibly used for antibiotics we performed many tests. The first thing we did was collect soil using the proper tools from Grafton Family Dental and Oconto Falls Dental LLC. We chose to compare dental offices to see which one has cleaner surroundings. Then we brought the soil to class where we performed a soil dilution and placed a small amount on a streak plate. After we let it grow, we chose different media to test our soil against what would be more similar to the environment that we took it from. The next tests that we did were master/tester plates, ESKAPES, colony PCR, and gel electrophoresis. The results of the ESKAPES showed clearing against two on the Grafton plates and two on the Oconto Falls plates. In conclusion, our hypothesis is correct because the bacteria that grew on the Grafton plates had more antibiotic-producing properties than the bacteria from Oconto Falls.

45 Dirt Doing the Hard Work: Discovering Antibiotics

KALIA VANG, ANGELO ROSSETT
University of Wisconsin - Green Bay

Antibiotic resistance is an international medical emergency. Tiny Earth research studies antibiotic-producing bacteria that reside in soil to combat this issue. A sample was collected from a garden, serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Five isolates were picked and patched to LBA to create master plates. Isolates were screened for antibiotic activity by patching them to LBA, Tryptic Soy agar (TSA), 10% TSA, LBA and potato dextrose agar (PDA) plates containing either *Escherichia coli*, or *Staphylococcus aureus*. Isolate 2 and 5 demonstrated antibiotic activity against *E. coli* and *S. aureus* on LBA, TSA, and PDA agar.

46 Tiny Earth: Diversity of Waterways vs Wooded Area

LCHELLE JOHNSON, KARLI CHAUDOIR, SHANNON GARDNER
Northeast Wisconsin Technical College

Bacterial pathogens are the cause of many deadly illnesses. Luckily, with the discovery and research of antibiotics, many have treatments and cures. However, antibiotic resistance is on the rise, and pathogens are learning how to evade the current treatments. This project aims to find antibiotic-producing bacteria in our own city! The three researchers tested for and found bacteria from 3 different sites: One from a creek bed in the north woods of Wisconsin, one from the shore on the Bay of Green Bay, and one in the woods of their own backyard. Through DNA testing, biochemical tests, and testing against the relatives of pathogens, the bacteria were tested for antibiotic-producing activity and identified. We have found that the high-traffic area of the Bay of Green Bay had more antibiotic-producing bacteria than that of isolated areas of an isolated creek and a secluded wooded back yard The isolated creek and wooded backyard did not show any antibiotic-producing activity. The Bay of Green Bay showed plate clearing against *B. subtilis, M. smegmatis* and *S. epidermidis*. If the discovery of a new bacteria comes from the project, there is a chance that a new antibiotic or class of antibiotics could come from these bacteria.

47 Soil and Sediment Samples had a Similar Number of Antibiotic-Producing Bacteria

KEVIN KYAW
Beloit College

The fight against antibiotic resistance has accelerated the need for effective antibiotics. Currently, two-thirds of our antibiotics come from *Acinetomycetes* that are primarily found in soil. This research focused on freshwater sediment as a novel environment to find antibiotic-producing bacteria. Sediment is influenced by aquatic environmental conditions and was hypothesized to contain antibiotic-producing bacteria that differ from those found in soil. Sediment and soil samples were collected from local parks with water systems around Beloit, Wisconsin, and plated on tryptic soy agar to culture bacteria. These colonies were screened against tester bacteria with similar characteristics to antibiotic-resistant bacteria to find effective antibiotic-producing isolates. Nine antibiotic producers were found in soil samples and ten were found in sediment samples. Further genetic analysis of the antibiotic-producing bacteria showed that the soil samples generally contained *Bacillus* and *Pseudomonas genera*, with sediment samples additionally including the *Planococcus* genus. In conclusion, antibiotic producers were found in both soil and sediment. Future studies will continue to explore the prospect of sediment as a novel source for antibiotic-producing bacteria. Freshwater sediment around Beloit, Wisconsin, will be used to find antibiotic-producing bacteria that will be further analyzed via genetic sequencing and the antibiotic metabolite will be chemically identified.

48 Testing High-Priority Antibiotic-Producing Isolates for Efflux from Escherichia Coli

ROSE MONTANYE
Janesville Research Institute

I have been testing high-priority antibiotic-producing isolates for their ability to inhibit the growth of different strains of *E. coli*, each which lacks a different efflux pump. I have been doing this with modifications to the disk diffusion assay. I have found more inhibition with *tolC* and *yohG* clones than wild-type *E. coli* for some TE isolates. This indicates *TolC* and *YohG* may be involved in pumping the antibiotics produced by the TE isolates out of cells. I believe this work can help us find ways that we can make these new antibiotics even more effective.

49 Pseudomonas Found on Tree Bark Produce Antibiotics Against Five Tester Bacteria

CAYMEN HOFFMAN
Beloit College

Bacteria compete for resources and space in their environment and some bacteria can produce antibiotics to give them a competitive advantage. Antibiotics are produced as a defense mechanism that kill or inhibit the growth of bacteria, but not all antibiotics are effective against every bacteria. Certain bacteria have protection from antibiotics in the form of antibiotic resistance and this resistance is causing the need for new antibiotics in the healthcare industry. The aim of this research was to identify the suggested genera of antibiotic-producing bacteria from seven types of tree bark (Alder, Burr Oak, Common Cottonwood, Eastern White Pine, River Birch, Sugar Maple, White Ash) and determine if the characteristics aligned with the suggested bacteria. Tree bark samples were collected from five locations in Wisconsin (Avon Bottoms Wildlife Area, Big Hill Park, Devil's Lake, Fischer Creek Park, Turtle Creek Greenway) and grown on three types of media (ESA, LBA, 50% TSA). All samples were screened against nine tester bacteria that represent various pathogens, including safe-ESKAPE relatives. Isolates that showed antibiotic production were sequenced using 16S rRNA gene sequencing and further characterized with biochemical tests. A variety of genera represented the antibiotic-producing bacteria on bark samples, although a majority of isolates were suggested to be *Pseudomonas* and confirmed as Gram-negative bacillus. More antibiotic production was prevalent on screens using the nutrient rich media ESA or 50% TSA. In conclusion, the suggested genera of antibiotic-producing bacteria aligned with the characteristics shown in the Gram stain, motility, and fermentation tests."

50 Effect of Grapefruit Seed Extract on the Prevalence of Antibiotic-Producing Isolates

CAROLINE VANDRISSE, CHARLIE HALASKA, JACKSON BANKS, TRISTON BEHREND
University of Wisconsin - Madison

The escalating global crisis of antibiotic resistance, fueled by the misuse and overuse of antibiotics, highlights the need for urgent efforts in antibiotic discovery. One method in antibiotic discovery is to adjust the conditions in which bacteria grow by treating the agar with grapefruit seed extract (GSE), a common natural antimicrobial agent, to examine the impact on antibiotic production of soil bacteria. Using soil samples to isolate bacterial colonies, colonies were screened against ESKAPE pathogen relatives to determine which colonies produced antibiotic compounds. A p-value of 0.95 revealed that there is no statistically significant relationship between colonies that produce antibiotic compounds and treatment of growing colonies with GSE. While GSE did not effectively impact antibiotic production of soil bacteria, there are numerous other antimicrobial agents that can be further studied.

51 Secrets in Our Soil

LIANNA THAO, EMILY SCHILLING
University of Wisconsin - Green Bay

Antimicrobial resistance is a global health crisis resulting from microbes like bacteria, viruses, and parasites no longer responding to the drugs available to treat infections caused by them. Our research addresses the crisis by studying antibiotic activity in soil bacteria. Throughout this research project, many tests were conducted to help identify and learn more about microbes in a soil sample collected from Menominee Park in Oshkosh, WI [next to Lake Winnebago]. Serial dilutions of a soil sample from Menominee Park were plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate bacteria. Isolates were placed on LBA and potato dextrose agar (PDA) to create master plates. Several colonies were patched to 10% tryptic soy agar (TSA), 100% TSA, PDA and LBA containing *E. coli* or *S. aureus*. Two isolates demonstrated antibiotic activity against *S. aureus* on 10% and 100% TSA plates. Future studies will determine if these antibiotics are useful for clinical use.

52 Antibiotic Resistance in Your Own Backyard

ALISON HAHN, EMILY BALFE
University of Wisconsin - Green Bay

More research needs to be done to resolve the crisis of antibiotic resistance. One way of doing this is to test soil for antimicrobial resistance. Soil is a rich source of diverse antibiotic-producing bacteria. A soil sample was collected from the Coffin Trail on the campus of UWGB (44.53711°N, 87.91666°W), serially diluted in sterile water and placed on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Approximately six isolates were patched to LBA and potato dextrose agar (PDA) to create master plates for experimentation. Isolates were patched to LBA, PDA, Tryptic Soy agar (TSA) and 10 % TSA plates to screen for antibiotic activity against *S. aureus* and *E. coli.* Isolate numbers three and four demonstrated antibiotic activity against *S. aureus* on TSA and LBA plates.

53 Deviant Critters: Uptick in Antibiotic Resistance

KELSEY THURIN, JONAH ZUEHLS
University of Wisconsin - Green Bay

Humanity has made tremendous progress in the field of medicine within the last century. Countless lives have been spared thanks to the deployment of antibiotics, however, our well of success is beginning to run dry due to antibiotic resistance. Tiny Earth research allows students to address the world crisis of antibiotic resistance by studying antibiotic activity by soil bacteria. A soil sample was collected from Bay Beach Sanctuary from the water bank, serially diluted in sterile water, and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Soil isolates were screened for antibiotic activity against *S. aureus* and *E. coli* on various media. Isolates were identified through an array of biochemical tests and gram-stain analysis BLAST analyses were conducted to obtain accurate identifications for each isolate.

54 Digging Deep on a Dirt-y Public Health Crisis

KAITLYN OTTE, MADDISON ERDMAN
University of Wisconsin - Green Bay

There is a dire need for novel antibiotics with bacteria becoming resistant to current treatments. Tiny Earth research addresses the dwindling supply of antibiotics through the isolation of antibiotic-producing soil bacteria. Our sample was collected from the UW-Green Bay campus (44.53460°N, 87.92956°W) and serially diluted in sterile water, then plated on Luria-Bertani agar (LBA) plates containing cycloheximide and incubated at 28 degrees Celsius for 48 hours. Following incubation, master plates were created by patching soil isolates on LBA and potato dextrose agar (PDA) plates. Isolates were screened for antibiotic activity by patching soil bacteria on LBA, PDA, Tryptic Soy agar (TSA) and 10% TSA plates containing *Staphylococcus aureus (S. aureus)* or *Escherichia coli (E. coli)*. Isolate two shows antibiotic activity against *S. aureus* on PDA and 10% TSA plates. This isolate is further characterized as gram-negative cocci. The antibiotic-producing bacterium we identified will be studied for the development of novel therapeutics.

55 The Basis of Antibiotic Discovery in Outagamie County

SAMI NEUBERT, ALLI HARTJES
University of Wisconsin - Green Bay

It is well known that antibiotic resistance is on the rise and many disease-causing bacteria are becoming untreatable. The idea of discovering new antibiotics to combat this resistance has also proven challenging with the current soil crisis. Our Tiny Earth project attempts to combat this rising issue by discovering new bacteria from a geographic location that has not yet been sampled and could contain untested rich antibiotic-producing soil bacteria. A soil sample was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Nine isolates were picked and patched on LBA and Potato Dextrose agar (PDA) to create master plates. Isolates were patched on LBA, PDA, 10 % Trypticase Soy agar (TSA) and 100% TSA plates containing either *S. aureus* or *E. coli* to screen for antibiotic activity. One soil bacterium showed antibiotic activity against *S. aureus* on all media utilized in this study.

56 Uncovering Antibiotics One Soil Sample at a Time

LIZ WILLIAMS, MAXINE VANG
University of Wisconsin - Green Bay

Tiny Earth research addresses the global crisis of antibiotic resistance by discovering new antibiotics produced from soil bacteria. A soil sample was collected from Lily Lake, Wisconsin with the coordinates of 44.42842 degrees N and 87.85396 degrees W, on September 9, 2023. One gram of soil was serially diluted in sterile water and placed on Luria-Bertani agar (LBA) plates containing cycloheximide to isolate soil bacteria. The cell density was calculated at 5.3 * 107 CFU/g. Master plates were created by patching eight soil isolates to LBA and potato dextrose agar (PDA) plates. Isolates were screened for antibiotic activity by placing them on LBA, PDA, Tryptic Soy agar (TSA), and 10% TSA plates containing *Staphylococcus aureus (S. aureus)* or *Escherichia coli (E. coli)*. Sample seven demonstrated antibiotic activity on 10% TSA-containing *E. coli*. PCR testing with BLAST analysis was performed on isolates 2 and 7. Isolate 2 is a gram-positive rod-shaped identified as *Metabacillus* (E-value of 925 with an 85.71% match). Isolate 7 is a gram-negative rod and bacilli-shaped identified as *Pseudomonas* (E-value of 0 with a 100% match). The findings will hopefully aid in slowing the spread of antibiotic-resistant infections.

57 Solving Global Antibiotic Resistance Through Researching Antibiotic Production in Soil Bacteria

CAMI FIGLINSKI, NOAH SCHAAF
University of Wisconsin - Green Bay

Tiny Earth attempts to solve global antibiotic resistance through student research by studying antibiotic production by soil bacteria. A soil sample from Luxemburg, Wisconsin was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. All isolates were screened for antibiotic activity against *E. coli* and *S. aureus*. One isolate showed antibiotic activity against *E. coli* on LBA as well as *S. aureus* on LBA.

58 Novel Antibiotics Unearthed

DESAHNI THAO
Green Bay West High School

Tiny Earth research addresses the global crisis of the decreasing amount of useful antibiotics. Our research is focused on discovering soil bacteria capable of producing novel antibiotics. We sampled soil specimens at the UW-Green Bay campus (44.5299373, -87.9211463). A soil sample was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide, which inhibits fungi, to separate soil bacteria. We picked and patched isolates to create master plates of the unique soil colonies. We then screened for antibiotic activity against *S. aureus* and *E. coli* on LBA, potato dextrose agar (PDA), tryptic soy agar (TSA), and 10% TSA plates. I was able to identify antibiotic activity with isolate 1 and isolate 2, both of which display broad-spectrum antibiotic activity against *S. aureus* and *E. coli*. We gram-stained the soil isolates to find the gram reaction and the morphology of the soil isolates. Future implications are to discover our soil antibiotic identity through the use of BLAST analysis and Colony PCR methods. We will send our isolates to Tiny Earth HQ for additional study and identification of the chemical structure of the antibiotics characterized by this study.

59 Antibiotic Discovery in Soil

NIKKI XIONG
Green Bay West High School

Tiny Earth research studies antibiotics produced by soil bacteria. A soil sample was collected from the UW-Green Bay campus (44.53050 N, -87.91950 W) and serially diluted in sterile water and plated in the presence of cycloheximide to isolate soil bacteria. We picked and patched the isolates onto Potato dextrose agar (PDA) and Luria-Bertani Agar (LBA) to create master plates. Isolates were screened for antibiotic activity by placing them on LBA, PDA, TSA, and 10% TSA containing *E. coli* or *S. aureus*. Isolate four showed antibiotic activity against *E. coli* on PDA agar. Isolate four also shows antibiotic activity against *S. aureus* on TSA and LBA. In conclusion, isolate four displays broad-spectrum antibiotic activity by inhibiting the growth of gram-positive and gram-negative bacteria.

STUDENT POSTERS

60 Uncovering Antibiotic Activity in Soil Bacteria

LAXAMEE VUE
Green Bay West High School

Antibiotic resistance is a global health crisis. Tiny Earth research addresses the crisis by examining antibiotic activity in soil bacteria. A soil sample collected on the UW- Green Bay Campus (44.5298097, -87.920495), was diluted in sterile water and plated on Luria-Bertani agar (LBA) plates. We created master plates by picking and patching the isolated bacteria to Potato Dextrose agar (PDA) and LBA. We screened for antibiotic activity by patching the soil bacteria to LBA, PDA, Tryptic Soy Agar (TSA) and 10% TSA plates containing either *E. coli* or *S. aureus*. Two isolates had antibiotic activity. Isolate #2 displayed antibiotic activity against *E. coli* on TSA plates and *S. aureus* on 10% TSA plates. Future experiments will focus on studying the structure of antibiotics.

61 Rich Soil, Rich Antibiotics

ANA GARCIA
Green Bay West High School

The world is running out of useful antibiotics. My assigned research is being done to discover new antibiotics that are produced by soil bacteria. We began this research by collecting soil from the UW-Green Bay campus (44.5297937, -87.9205068). A soil sample was serially diluted in sterile water and plated on Luria-Brentani agar (LBA) containing cycloheximide to isolate soil bacteria. We patched our soil bacteria on potato dextrose agar (PDA) and LBA to create master plates. These plates provided a source of material for various experiments. Isolates were transferred to LBA, PDA, Tryptic Soy agar (TSA) and 10% TSA plates containing *Escherichia coli* or *Staphylococcus aureus* to screen for antibiotic activity. Isolate #1 had antibiotic activity against *S. aureus* on LBA and 10% TSA. Isolate #1 was gram-stained and we observed that the results were gram-positive, bacilli (rods). We amplified the 16S RNA gene (molecular fingerprint) of isolate #1 by PCR and will use BLAST analysis to identify the genus of the bacterium. Isolate#1 was subjected to biochemical tests, including catalase, blood agar, gelatin tubes, starch agar, motility, SIM, Thioglycolate, and MacConkey agar to confirm the molecular identity of isolate#1.

62 The Antibiotic Odyssey: Deep into the Ground

JESSENIA CRUZ
Green Bay West High School

The world needs new antibiotics to address the crisis of antibiotic resistance. Tiny Earth research studies antibiotic activity in soil bacteria. A soil sample was collected on the UWGB campus (44.529790, -87.920571), serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. The bacteria isolates were patched to Potato Dextrose Agar (PDA) and LBA plates, creating master plates for additional experimentation. Isolates were patched to LBA, PDA, Tryptic Soy Agar (TSA) and 10% TSA plates containing either *E. coli* or *S. aureus* to screen for antibiotic activity. Isolates 1, 3, and 4 displayed antibiotic activity against *S. aureus* on LBA and both TSA agar plates. During the gram staining process, isolates 1 and 3 were gram-positive bacilli and isolate 4 was gram-negative cocci.

63 The Unveiling of Antibiotics Through Microbiology

CHLOE BURTON
Green Bay West High School

Tiny Earth research studies antibiotic activity in soil bacteria to address the crisis of antibiotic resistance. One gram of soil was serially diluted in sterile water to separate bacteria from our soil sample. We created master plates through picking our isolates from the diluted agar plates and patching them to Luria-Bertani Agar (LBA) plates. We screened for antibiotic activity by placing soil isolates on Potato Dextrose Agar (PDA) plates, Tryptic Soy agar (TSA) plates, 10 % TSA plates, and LBA plates containing either *Staphylococcus aureus (S. aureus)* or *Escherichia coli (E. coli)*. All of my isolates displayed broad-spectrum antibiotic activity. Isolate #1 is a gram-negative bacillus. Isolate #3 is a gram-negative coccus with spores. Isolate #4 is a gram-negative bacillus.

64 Antibiotic Discovery: An Odyssey of Combating Growing Resistance

BRENNA RUONAVARRA
Green Bay West High School

Tiny Earth is aiding the global search for more antibiotic-producing bacteria to help fight antibiotic resistance, a global health emergency. After picking and patching six unique soil isolates to potato dextrose agar (PDA), Luria-Bertani agar (LBA), tryptic soy agar (TSA), and 10% TSA plates containing *E. coli* or *S. aureus*, I have found that I have three antibiotic-producing isolates. Isolates #2, #3, and #5 demonstrated antibiotic activity against *Staphylococcus aureus* on TSA plates. Isolate #2 also showed antibiotic activity against *E. coli* on TSA, and *S. aureus* on LBA. Isolates #3 and #5 showed activity against gram-positive bacteria only, demonstrating narrow-spectrum activity, while isolate #2 displayed broad-spectrum activity, as it works against gram-negative and gram-positive bacteria. All three soil isolates were gram-positive bacilli. This research is important because it helps us understand how microflora function in our efforts to improve human life. In the future we will focus on identifying the chemical structure of these antibiotics, and how we can apply the information to our current global research!

65 Antimicrobial Activity of "B10" Against S. epidermidis

GRACE THOMAS-HANEY
Janesville Research Institute

As the antibiotic crisis continues, many of our commonly used antibiotics turn ineffective after repeated use. This leaves the medical community with limited options, which includes the search for novel bacteria that can produce antibiotics against infectious diseases. Through field samples of soil from various locations, the search for a bacteria with antimicrobial properties against various ESKAPE pathogens began. From Kiwanis Pond (Janesville, WI), an isolate dubbed "B10" was tested against lawns of *Enterobacter aerogenes, Escherichia coli, Pseudomonas putida, Acinetobacter baylyi, Bacillus subtilis* and *Staphylococcus epidermidis*. A zone of inhibition was produced when "B10" was placed against a lawn of *Staphylococcus epidermidis*. After repeated plates, it could be reasonably concluded that "B10" possesses antimicrobial activity against *S. epidermidis*. Biochemical and molecular tests have been performed to determine the identity of "B10".

66 Effects of Culture Media Concentration on Antibiotic Production in Soil Bacteria

AARON NOBLE, JOSHUA CRUZ
Illinois Valley Community College

Shortly after the discovery of antibiotics, certain bacteria began to show resistance to these new medications. This created a rush as researchers began to look towards antibiotic-producing soil bacteria for new drugs to combat this resistance. While some soil bacteria will grow in the lab, they may change phenotypes, and lose traits such as antibiotic production. This could be happening because they are being grown on nutrient-rich media as opposed to their natural environment. We hypothesized that soil bacteria grown on a lower concentration of culture media would produce more antibiotics than if they were grown on a full-strength media. We selected seven unknown bacterial strains that had shown antibiotic activity against one or more of our test pathogens. We made lawn plates using both standard Yeast Malt Extract Agar as well as a 10% concentration of the same media. These strains were incubated for five days at 28°C. Assay extracts were made from these plates using methanol. The extracts were then tested against the species they previously showed activity against to see if there was a difference in antibiotic production by bacteria grown on the different media. Our results have been inconsistent. One strain showed better activity when grown on the fully concentrated media. Other strains showed little activity when compared to methanol controls, so it was hard to determine what effect, if any, the different concentrations of media were having. We have concluded that there is not a one-size-fits-all solution for inducing better antibiotic production from soil bacteria.

67 Root Microbiota of Lavender and Oak Plants: Comparison of Antibiotic Production

KATHERINE FEICK, ANI LANGSETH, CLAIRE CHOI, ANTHONY ARNHOLT
University of Wisconsin - Madison

Antibiotic resistance is a looming crisis which could affect millions worldwide if left unaddressed. With this chronic increase in antibiotic resistance, research is being done on the microbes found within the soil of the Earth, testing to see which microbes could potentially lead to the next antibiotic discovery. Many medicinal plants, such as lavender, are often prescribed as potential at-home remedies to beat common illnesses such as the common cold, due to their antimicrobial properties. Therefore, it is possible that a relationship between host plants and root microbiota exists in which these medicinal plants can confer their antimicrobial traits to their root microbiota.This experiment was designed to discover the relationship between microbes taken from natural medicinal and non-medicinal plants to answer the question: how do antibiotic properties in root microbiota of medicinal and non-medicinal plants compare? It was determined that there is no relationship between the effectiveness of a particular root microbiota in producing antimicrobial active bacteria. That being said, future studies should continue to research good sources of microbial compounds that are readily available, diverse, and culture-dependent to provide greater information for the development of new drugs against antibiotic-resistant strains.

68 Understanding the Threat of Antimicrobial-Resistant Bacteria: A Global Health Crisis

KAITLYN MONTAG, HAILEY HECKENLAIBLE
University of Wisconsin - Green Bay

A problem in our world today is antibiotic resistance. The research conducted in this project addresses antimicrobial-resistant bacteria by analyzing bacteria in a soil sample obtained from Wequiock Falls located in Green Bay, WI. One gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Using the "pick and patch" method, we created master plates on Potato Dextrose agar (PDA) and LBA. The soil sample from Wequiock Falls offered a diverse population of bacteria. Isolates 2, 4, and 6 produced metabolites inhibiting the growth of *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* on PDA and 100% Trypticase soy agar (TSA) plates. Our findings will be put into a database of soil samples from around the world to inform others of the presence of antibiotic-producing strains of bacteria. This can be important for developing new antibiotics, and subsequent research in the field of antibiotic discovery.

69 Antimicrobial Effects of Small Molecule Acids and Indoles

SYDNEY MEIER
St. Norbert College

This research aims to determine the antimicrobial activity of compounds used in Rockline Industries' disinfectant wipes. Previous work centered around the antimicrobial effects of ColaLipid C (CL) and other surfactants, along with several organic acids. The current study analyzes small molecule acids and indoles with poor water solubility using non-CL solvents that do not inhibit bacterial or fungal growth alone. The research used the ESKAPE bacteria, the safe relatives of clinically relevant bacterial pathogens, along with several fungi. The bacteria were grown in Mueller-Hinton broth, diluted, and distributed into a 96-well plate with the respective testing solution. Growth inhibition was measured at 24 or 48 hours using a microplate reader. Fungi were grown on YM plates and plugs containing mycelia were placed into 6-well plates with the respective testing solution for 24 hours. The solutions were plated on fresh YM plates, incubated, and fungal colonies were counted. Many individual organic compounds and combinations of solvents and compounds were tested. A select few were successful in inhibiting bacterial and/or fungal growth. Future plans include testing solution parameters like pH, additional organic acid and indole combinations, and testing more fungal species.

70 Exploration of Endophytic Species from Wisconsin Towards the Discovery of New Natural Product Antibiotic Scaffolds

LILIA SHALLOW, GRACIE ROHR
St. Norbert College

Bacterial resistance to antimicrobial agents has been a problem since the first antibiotic was introduced in the 1930's. However, this issue is becoming significantly more urgent as the number of multidrug-resistant microorganisms increases. Compounding the problem, most antibiotics approved since 1970 consist of iterations of previously discovered structures; only four new scaffolds have been introduced since that time. There is an obvious need for new antimicrobials. To locate potential novel natural product antibiotics, we are focusing our research on a class of organisms called endophytes. Endophytes are bacteria and fungi that colonize the interior organs of a host plant without causing pathogenic effects. The unique plant-endophyte relationship is maintained by a complex secondary metabolome that has been evolutionarily optimized for biological efficacy. While not always the case, colonization by these organisms is often beneficial to the host plant. For example, endophytes can enhance the stress tolerance of the host plant through the production of compounds that combat plant predators including bacteria, potentially privileging them over other natural products in the ability to serve as antimicrobials. The evidence suggests that endophytic microorganisms offer a largely unexploited abundance of natural products. Despite this potential, little energy has been focused on their study until quite recently. We are hoping to exploit the paucity of information in the scientific literature regarding endophytes in Wisconsin to maximize our potential of finding novel organisms and, subsequently, novel antibiotic scaffolds. Additionally, we have focused our exploration on plants from unique local ecosystems, that are endemic, that are known to have an unusual longevity, or that have a reported ethnobotanical history; these types of plants are thought to be most likely to harbor a diverse set of endophytic organisms and, therefore, a diverse set of bioactive natural products. The endophytes isolated from our collected plant samples were co-cultured with several target pathogens to screen for the production of secreted secondary metabolites with antibiotic activity. Endophytes showing activity in these screens were identified using common microbiological techniques and 16S rRNA sequencing. Work on identifying the endophytes and the antimicrobial natural products they produce is ongoing.

71 How Do Changes in the Growth Environment Affect Antibiotic Production?

MANDISA TIMBA
North Park University

The rapid emergence of multidrug–resistant (MDR) bacterial pathogens pose a major threat to human health. Soil samples were collected from various locations within the Chicago Park District lands. The One Strain Many Compounds (OSMAC) tool underlines how a single strain can produce different molecules when grown under different environmental conditions. Parameters such as nutrient content or source, temperature, and chemical composition can be easily changed, altering the physiology of a microbial strain and in turn, significantly affecting its secondary metabolism. Bacteria require basic nutrients such as carbon and nitrogen for their metabolic functions altering the concentration of these nutrients will introduce a stressful environment which will create different transcriptional patterns.

72 Nuclear Soil: Antibiotic Discovery in a Unique Location

ALLI MISIALEK, ELLA ANDERSON
University of Wisconsin - Green Bay

Antibiotic resistance is increasing dramatically. Tiny Earth research focuses on antibiotic discovery by studying soil bacteria. A soil sample was collected on the border of the Point Beach Nuclear Power Plant about 1.5 inches below the surface. One gram of soil was serially diluted in sterile water and spread on four Luria-Bertani agar (LBA) plates containing cycloheximide to isolate soil bacteria. Isolates were patched to LBA and Potato Dextrose agar (PDA) to create master plates. They were then screened for antibiotic activity by patching them to LBA, PDA, Tryptic Soy agar (TSA), 10% TSA plates containing either *S. aureus* or *E. coli*. One isolate displayed antibiotic activity against S. aureus on the TSA 10% plate, creating a zone of inhibition. With this sample of soil being taken from a unique location, there is a strong possibility that the bacteria discovered will contain antibiotic activity and will be a step towards an expansion of the antibiotic library.

73 Antimicrobial Activity of Leaf Extracts of Veronicastrum virginicum

ISABELLE ARNOLD
St. Norbert College

Veronicastrum virginicum is a plant native to the eastern United States historically known for being used by Native American tribes for medicinal purposes. Other members of its family have also been used medicinally and shown to produce phytochemicals, including iridoids, which are known to have antimicrobial activity. *V. virginicum* has been looked into for its antimicrobial activity because of the secondary metabolites it contains. We hypothesized that *V. virginicum* extracts include chemicals that inhibit the growth of clinically relevant microbial pathogens. We tested *V. virginicum* extracts for antimicrobial activity using disc diffusion assays against various ESKAPE pathogens. Preliminary results suggest that antimicrobial properties are present as a zone of inhibition appeared around the leaf extract sample with *Staphylococcus epidermidis* bacteria. Further work will be able to identify the bioactive compounds within the leaf extract and at what varying concentrations does the antimicrobial activity become visible with this bacteria.

74 Antibiotic-Producing Bacteria Found in Southern Wisconsin Water

EDGAR CARACOZA
Beloit College

Soil erosion can drastically change the future of healthcare because most known antibiotic-producing bacteria are found in soil. The number of antibiotic-resistant bacteria are increasing as antibiotics continue to be misused, which leads to the research question of where to find novel antibiotic-producing bacteria besides soil. This research identified antibiotic-producing bacteria from around the Wisconsin Dells and Beloit, Wisconsin. The methods included collecting water samples, plating serial dilutions on Luria broth, nutrient agar, and 50% nutrient agar, and culturing bacteria with diverse colony morphologies. Then, select isolates were screened against tester bacteria, sequenced to determine the 16S rRNA, and biochemical tests were conducted to further classify the isolates. Approximately 8% of the total isolates tested produced antibiotics. The ten isolates identified as antibiotic-producing bacteria had cell morphologies of bacillus or coccobacillus and were cultured from various water sites in Southern Wisconsin. More specifically, four of the ten isolates were from the Wisconsin River. Nine of the ten isolates were Gram negative with five of the bacteria fermenting lactose. The most prominent genera identified were *Chromobacterium* and *Pseudomonas*. In conclusion, water may be a potential source of antibiotic-producing bacteria. Future studies will extract the antibiotic metabolites from these microbes.

75 Identification and Characterization of Microbial Organisms in Drosophila Melanogaster Exposed to Tetracycline

SAMANTHA PARDINI
St. Norbert College

The identification and characterization of microbes that colonize different microbiota is a growing field of interest as microbial dysbiosis has been attributed to several types of diseases including gastrointestinal diseases like Crohn's and Irritable bowel syndrome as well as neurodegenerative and pulmonary disease (Manos 2022). As demand continues to rise for research into the gastrointestinal microbiota, there is a need for an efficient and easy-to-handle model system. Drosophila melanogaster offers a simpler gastrointestinal tract than traditional mammal model systems, and it has similar properties to the human microbiota (Ludington & Ja 2020). In addition to being cheap and easy to rear, germ-free or microbe-free flies can be created and maintained and the microbiome can be modified by exposure to antibiotics and environmental factors. Thus, the *Drosophila melanogaster* model system is ideal for future investigations of the gastrointestinal microbiota. In this experiment, the internal microbiota of *Drosophila melanogaster* was characterized with several different biochemical tests and 16S rRNA sequencing. The initial test results suggest that the organism can ferment disaccharides, is non-motile, and is a facultative anaerobe. The identification will be confirmed with 16s rRNA sequencing results. The data presented here demonstrate how individual members of the *Drosophila melanogaster* microbiota can be isolated and characterized. This will be useful for future studies that investigate the interactions that are mediated by these organisms and the overall impact on the flies.

76 Soil Solutions: Earth's Antibiotic Arsenal

JORDAN SOPJES
University of Wisconsin - Green Bay

Antibiotics help humans fight pathogenic bacteria. However, antibiotic resistance has become a global health crisis. Therefore, new antibiotics are required to combat antibiotic resistance. Using a soil sample collected near the UW-Green Bay campus (44.5313° N, 87.9210° W), one gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria in the hopes of identifying novel antibiotics. Master plates of separated bacteria were patched to LBA and potato dextrose agar (PDA) for semester-long experimentation. Isolates were screened for antibiotic activity by patching isolates from master plates to LBA, PDA, Tryptic Soy agar (TSA), and 10% TSA plates containing E. coli or *S. aureus*. The implications of this research have the potential to be substantial. Given the increasing antibiotic resistance, the project aids in finding new antibiotic candidates, potentially addressing a global health crisis.

77 Soil Isolate Antibiotic Producer

ELLEIGH CASPER, SADIE OLEJNICZAK
University of Wisconsin - Green Bay

Antibiotic resistance is a serious health concern. Bacteria are becoming resistant faster than antibiotics can be produced. Testing soil bacteria for antibiotic-producing properties is a viable option for reducing the impact of antibiotic resistance. A soil sample was collected from a residential area in Algoma, serially diluted in sterile water, and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. Master plates containing different isolates were created for further testing. The isolates were then screened for antibiotic production by inoculating Tryptic soy agar (TSA), 10 % TSA, potato dextrose agar

(PDA), and LBA containing either *Escherichia coli* or *Staphylococcus aureus*. One isolate on LBA was identified to be a potential antibiotic producer against *Staphylococcus aureus*. Polymerase chain reaction was then completed to obtain enough 16S rRNA for sequencing and identification of the isolate.

78 Impact of Manuka Honey on Antibiotic Production in Soil Bacteria

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Antibiotic resistance represents one of the greatest challenges in modern medicine, posing a significant threat to public health and overall human well being. This study aimed to investigate the impact of the antimicrobial properties of manuka honey on the antibiotic-producing potential of soil bacteria. To examine the effects of manuka honey as a culture additive on the antibiotic-producing potential of soil bacteria, bacterial colonies were cultivated under various concentrations of manuka honey, and were then tested against the safe relatives of antibiotic-resistant human and plant pathogens. Our results indicated a significant, positive relationship between high concentrations of manuka honey in bacterial cultures and the proportion of antibiotic-producing bacteria. This finding underscores the importance of exploring novel and inventive culturing techniques in the search for new antibiotics to combat the growing threat of antibiotic resistance.

79 Niacin Impact on Antibiotic Production in Soil Microbes

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The antibiotic crisis is at the forefront of modern medicine, as antibiotic discovery has been nearly stagnant over the last few decades. The impact of vitamin treatments on soil bacteria is understudied, making it a desirable area to look for possible antibiotic production. Niacin is a water-soluble organic compound, vitamin B3, important in growth and metabolic activity. This study focused on how niacin would affect antibiotic production in soil bacteria collected from the campus area of Madison, WI. A control and experimental group, treated with 1g/10ml of niacin, were screened against five safe ESKAPE relatives to test for the presence of antibiotic compounds via zones of inhibition. Results showed the difference between the groups to be insignificant. Although this study indicates that niacin does not induce antibiotic production, it may be beneficial to conduct future research using other vitamin treatments.

80 Analyzing Antibiotic Production of Soil Bacteria in the Presence of Irish Sea Moss

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The rise of antibiotic resistance poses both a threat to public health and efforts in antibiotic discovery and production itself, inciting exploration into alternative methods of research. Sea algae is a naturally occurring compound with antibiotic properties with potential in the innovation of new antibiotics. This research investigates the question: How does the addition of Irish sea moss extract to soil microbes affect the growth of antibiotic-producing bacteria? This was conducted by first plating sea moss (a form of sea algae) and soil microbes at different concentrations of sea moss (10-2 and 10-4) and then screening the bacteria produced with ESKAPE pathogen safe relatives to determine antibiotic production in colonies. The antibiotic-producing colonies were then analyzed using PCR, BLAST analysis, and MacConkey agar to determine the classification of the bacteria and how it relates to current pharmaceuticals used to treat disease. Multiple antibiotic-producing colonies were observed, however, there was no observed statistical difference between sea algae plates and control plates suggesting that sea moss does not have an effect on the growth of antibiotic-producing bacteria.

81 Antibiotic-Producing Bacteria in the Rhizospheres of Medicinal Plants: Examining the Broadleaf Plantain (Plantago major)

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The current crisis of antibiotic resistance poses a severe global health risk, as the rate of antibiotic discovery falls far below the demands posed by increasingly resistant pathogenic bacteria. Medicinal plants have a long history of supporting human health, including the broadleaf plantain *(Plantago major)*, which is well known for its antimicrobial capabilities. Soil is home to diverse and abundant bacterial species, with especially interesting communities connected to the plant rhizosphere.

Rhizospheres of *Plantago major* and nearby control locations were sampled to determine which would yield more antibiotic-producing bacteria. Bacteria were cultured from the samples, then screened against safe relatives of antibiotic-resistant pathogens to determine their antibiotic-producing capacities. No statistical difference was found between the amount of antibacterial producers in either the control or *Plantago major* samples. Despite the lack of statistical significance found in the data, it is still important to address and research possible ways of solving the antibiotic crisis today.

82 Identification of Bacteria and its Secondary Metabolites from Unique Entisol

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The Conata Ranch, situated in South Dakota at the heart of the Conata Basin, is characterized by its unique soil conditions due to isolation and relatively unchanged soil composition for thousands of years. The soil type is predominantly from the Valent series, a young entisol no more than a few thousand years old. Valent soils are very deep, excessively drained soils formed in mixed eolian sands, found on upland dune topography. During the late Pleistocene, a period marked by significant climatic changes, it's plausible that these soils hosted unique microorganisms.These microorganisms not only play a crucial role in maintaining the area's biodiversity but may also produce specialized chemical compounds to compete for resources. Such compounds have potential applications as antibiotics, offering a solution to the escalating global challenge of antibiotic resistance.

Our hypothesis is that the distinct regions of Conata Ranch may yield novel microbes with the potential to produce unique antibiotics. To investigate this, we analyzed the collected soil samples for microorganisms and assessed their antibiotic production against ESKAPE safe relatives, which are akin to the most common drug-resistant pathogens causing hospital-acquired infections. Further, we plan to conduct chemical analyses of these compounds to determine their structure and composition. Collaborating with Tiny Earth, our goal is to identify microorganisms capable of generating new antibiotic substances.

In our recent findings, we have attempted to identify the bacteria obtained from the soil and test for the metabolites being produced by the bacteria. The bacteria identified with antibiotic activity against *S. epidermidis,* resembles *Bacillus thuringiensis*. Based on an analysis of the biosynthetic gene clusters of this organism, it appears that the organism is likely producing compounds that resemble petrobactin, thuricin, and zwittermicin A. In the future, we plan to run chemical analysis to determine the exact structure of the metabolites.

83 The Antibiotic crisis is not "Fungi"

ELIZABETH NAVARRO CASTAÑEDA, STEPHANIE MIRELES
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How long do we have until a common infection like Strep throat becomes deadly? The answer is not long. Unfortunately, we are facing an antibiotic resistance crisis, and we are running out of time. The "Tiny Earth" project has opened a door for us students to be part of the search for a new antibiotic. Our hypothesis was that if we collected soil in an area with mushrooms, we would have a higher chance of finding an antibiotic producing microbe since research has found many species of mushrooms with antimicrobial properties. We collected from two locations, Pamperin Park and Cofrin Memorial Arboretum Trail. First, we diluted the soil to reduce the concentration of our original sample to allow single colonies to grow. Second, we cultured our single colonies in different conditions; PDA, TSA, LB to see if the number of bacteria would increase. We tested for clearing on our master plate. We screened our microbes against *S. epidermidis* and *Erwinia*. Neither of our samples showed positivity against *Erwinia*. However, there was a clearing on both of our samples from *S. epidermidis*. This clearing meant that there was potential for an antibiotic producing microbe. Our research is still on going with genetic and biochemical testing.

84 *Pseudomonas frederiksbergensis* Shows Antimicrobial Activity Against *M. smegmatis*

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Soil bacteria are known to produce antibiotic compounds, some of which have been isolated and exploited as pharmaceuticals (Clardy 2009). However, soil still contains a large number of unclassified bacteria, with some soil types having up to1.86% of their bacteria not classified at the phylum level (Delgado-Baquerizo 2019). While most are likely to be found in tropical soils, North American temperate soils still harbor undescribed organisms. Some of these may be capable of secreting novel antibiotic compounds.

To explore this possibility, we obtained samples from two common, yet distinct, soil ecosystems in northeast Wisconsin. We isolated the resulting bacterial colonies and tested them against relatives of the ESKAPE pathogens. We then attempted to identify any colony which showed promise in inhibiting growth. Of those organisms, we noted one that showed antimicrobial activity ag1ainst *Mycobacterium smegmatis.*

This organism had been sampled from shallow garden soil in Little Suamico Township, WI. After sequencing its DNA, we were able to identify this bacterium as a species in the genus *Pseudomonas*. Unfortunately, our experiment did not identify any novel organisms. We would suggest that future efforts sample a wider diversity of soils, in eluding those found in more natural and balanced ecosystems, such as mature forest.

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