tiny earth

12|6|2024

in TITLE TOWN

LAMBEAU FIELD, GREEN BAY, WI

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tiny earth

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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 16,000 students per year who are contributing to student source new antibiotic discovery from soil. Tiny Earth is expanding rapidly throughout Wisconsin. To date, 48 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**
UW-Green Bay

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

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

» **Valerie Martin-Conley**
Chief Academic Advisor, Saint Norbert College

» **Kristen Raney**
President, NWTC

» Land Acknowledgement
Josh Besaw Sr.
Student, College of Menominee Nation

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

» Antibiotic Stewardship and Innovation
Laurel Legenza, PharmD, PhD
Research Scientist, Wisconsin School of Medicine and Public Health, University of Wisconsin - Madison

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.

Valerie Martin-Conley

Chief Academic Advisor, 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).

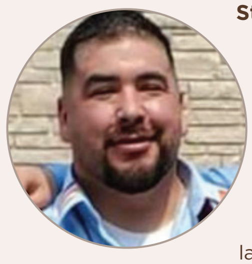
She earned her doctoral and master's degrees from Tulane University, and graduated magna cum laude with a bachelor's degree in sociology from Loyola University New Orleans, where she later served on the faculty and held administrative positions of increasing responsibility.

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 Northeast Wisconsin Technical College (NWTC), a finalist for the Aspen Prize for Community College Excellence. She is the first female and eighth president in NWTC's 112-year history. She previously served as the vice chancellor for academic affairs at Eastern Iowa Community Colleges, in Davenport, IA, and the vice president of academic affairs at Saint Paul College in St. Paul, MN. Dr. Raney's career began at Chippewa Valley Technical College (CVTC), where she served as faculty and dean. She is a Peer Reviewer with the Higher Learning Commission and an Aspen Presidential Fellow. Dr. Raney earned degrees from Edgewood College, University of Wisconsin-Stout, and St. Cloud State University.



Josh Besaw Sr.

Student, College of Menominee Nation

Posoh, my name is Joshua Besaw Sr., I am a student at the College of Menominee Nation. I will graduate in May of 2025 with an Associate degree in Business Administration. Upon graduation I will continue to study Natural Resources. I have been employed at Menominee Tribal Enterprises in Neopit, Wisconsin for the last 20+ years. I am currently the Sawmill Operations Manager. I am a Six Sigma green belt and have studied continuous development at North Central Technical College in Antigo Wisconsin. I graduated from the National Hardwood Lumber Association (NHLA), in Memphis Tennessee in 2005 to become a certified lumber inspector. I am a proud member of the Menominee Indian Tribe of Wisconsin.

Angelo Kolokithas

Tiny Earth Partner Instructor, NWTC

Dr. Angelo Kolokithas is the program director of biology at Northeast Wisconsin Technical College (NWTC) where he teaches courses in biology, microbiology, cell biology, and experimental design. He also directs retroviral research at the college. Before NWTC, he performed retroviral research at the National Institutes of Health. Dr. Kolokithas received his BS in cell and molecular biology from San Diego State University and his PhD from the University of Montana/National Institutes of Health partnership.

Laurel Legenza, PharmD, PhD

Research Scientist, Wisconsin School of Medicine and Public Health, University of Wisconsin-Madison

Laurel Legenza, PharmD, PhD is a Research Scientist at the University of Wisconsin School of Medicine and Public Health within the Department of Medicine. She is also Teaching Faculty and the Director of Global Health at the UW-Madison School of Pharmacy. Dr. Legenza received her PharmD from the UW-Madison School of Pharmacy and her PhD in Pharmacy from the University of the Western Cape, South Africa. She is passionate about improving patient outcomes, specifically addressing antimicrobial resistance with action-oriented data for treatment decisions. She leads research projects on antimicrobial resistance geographic mapping and developing clinical decision support tools for infectious diseases.

STUDENT POSTERS

1 Urban vs. Rural Soil Secrets

QUINCI TAPPA, MORGAN RUBRINGER
Northeast Wisconsin Technical College

Over many years, the problem of antibiotic resistance has become more significant due to scientists not being able to treat pathogens with already discovered antibiotics. With pathogens resisting current antibiotics, no new antibiotics have been discovered to help this crisis. The Tiny Earth program allows students to find possible antibiotics in the microbes of our soil samples chosen by the students. Our soil samples were taken from the rural area of Stephenson and urban area of De Pere. Both locations hypothesizing one antibiotic producer. We hoped to believe that the Stephenson sample would produce more due to less pollution and activity in a rural area. In the lab we diluted our samples to then plate using proper streaking techniques. From this point, we chose 20 bacteria of our liking and screened them on a plate against *S. epidermidis* and *Erwinia* to determine if we had any antibiotic activity. The Stephenson sample did not show any antibiotic activity as well as the De Pere sample. To further our study, we chose one bacterium to continue testing with. With these chosen bacteria we screened against the ESKAPE pathogens. As before, the Stephenson sample still had no showing of any antibiotic activity. However, the De Pere sample unexpectedly showed antibiotic activity against *Bacillus subtilis*. While the hypothesis for the Stephenson sample was not supported, the findings for the De Pere sample aligned with our expectations, demonstrating the potential of urban soils to harbor antibiotic activity. Our research for the antibiotic producing bacteria of the Stephenson and De Pere samples are ongoing with continued observations, testing, and results.

2 Relaxing and Chilling with Bacteria

PA VUE
Northeast Wisconsin Technical College

Scientists are finding it difficult to develop new antibiotics. These issues have resulted in increased mortality rates worldwide. The organization, Tiny Earth, provided an opportunity to explore soil in our communities in search of new microbes that produce antibiotics. This study involved collecting a soil sample from St. James Park for the Tiny Earth Project. St. James Park is a lovely green space located across from a church of downtown Green Bay, WI. What makes this park unique is its peaceful setting, surrounded by tall trees and frequented by playful squirrels running around. It's refreshing and very chilly due to having so many shades around it. The collection process involved using sterile sampling equipment to ensure the integrity of the samples. It is hypothesized that this sample will contain two antibiotic-producing microbes. The methods I used are serial dilution, culture conditions including PDA and dark plates, the creation of master & tester plates, an all-tester plate & streak plate. The only activity displayed was one clearing zone against the Gram-negative *Erwinia* plate. The process of collecting the soil sample was meticulous, ensuring that the sample represented the diversity of microorganisms present in the park. Despite the initial anticipation of finding multiple antibiotics-producing microbes, based on the results, it appears that I have fewer antibiotics than I initially predicted. This prompts further investigation into the microbial composition of the soil in St. James Park and the factors influencing antibiotic production.

3 The Dump That Saves The World

LOGAN BAERENWALD
Northeast Wisconsin Technical College

Based on the title, you may be thinking about defecation. Please reconsider, as there are lives at stake! Those lives being the ones impacted by new and improved 'super' bacteria. With the rise of antibiotic-resistant bacteria, to include the lack of antibiotic production, something needed to be done. Enter the Kewaunee dump, a dump so massive that it has the power to potentially save lives. The purpose of using the Kewaunee dump was earnest in nature, as it is a rough place to carry out an existence. I wanted hearty, tough bacteria who knew how to survive against the toughest of adversaries. By digging up the dirt at the Kewaunee dump, I hypothesized there would be "a lot" of antibiotic producing bacteria. In the lab, I used the soil dilution method, to further isolate bacteria. I also changed culture conditions, through the use of TSA and PDA plates. Finally, I isolated my good bacteria with the largest zones of clearing and pitted them against tiny adversaries. Through these methods, it was discovered that the massive Kewaunee dump did in fact have antibiotic producers. In conclusion, this was not as many as I initially thought, however, the final isolated bacteria had produced antibiotics. This antibiotic only worked against one tiny adversary, *B. subtillis*, a gram-positive, *bacillus*, strepto bacteria. This means that while my hypothesis wasn't necessarily totally correct, it wasn't incorrect either. Out of the 20 bacteria tested from the master plate, one had antibiotic producing qualities.

4 Stop and Smell the Flowers

BROOKLYN BADENDICK, TINA PETERSON
Northeast Wisconsin Technical College

The growing crisis of antibiotic resistance has put our health, economic, and political status at a higher risk than ever before. Scientists have worked tirelessly at helping to grow our future health system but have been bombarded by the negligence of today's society. The Tiny Earth organization has found a way to give the scientist a helping hand. Students at NWTC have the opportunity to collect soil from around the community in hopes of finding a new antibiotic-producing bacteria that can help fight antibiotic-resistance. In hopes of discovering a new antibiotic we took our samples from two different counties that have similar environments. One sample was taken from a local botanical garden and one from an organic flower farm. Our hypothesis has led us to the belief that a wider diversity of flowers will have a higher chance of producing antibiotics because of its rich nutrients and diverse species growth. We started our antibiotic journey by collecting our soil and diluting it in class. After diluting our soil, we plated our diluted soil samples onto three different plates. The plates contained bacteria including *Erwinia* and S.epidermidis plus an extra plate that we used as a master. The Botanical sample showed 2 signs of activity on *Erwinia* and 2 signs of activity on S.epidermidis. The organic sample on the other hand only showed 1 sign of activity on S.epidermidis. This means that our hypothesis was correct. Our soil research is still in progress, and more information will be determined soon.

5 Gardening Antibiotics

ALLISON PAHL, KYLA VAN REMORTEL, JENNY OVERMAN, CAILIN MORELAND
Northeast Wisconsin Technical College

Throughout our microbiology course, we have been learning about and identifying antibiotic-producing agents in hopes of isolating them and fighting back diseases caused by ESKAPE pathogens. With the help of the Tiny Earth Organization, we had the opportunity to excavate soil from four different agriculture-influenced gardens throughout Northeast Wisconsin to potentially discover antibiotic-producing agents that may aid in the current antibiotic crisis. We believe these locations will capture the public's interest as it is fascinating to consider the possibility of antibiotic-producing microbes growing right in our home gardens. With the significant availability of different resources present in each garden and the similarities among the sample sites, our group hypothesized each garden would produce anywhere from two to four antibiotic-producing microbes. Our methods included diluting and plating our soil from the four sample sites. These microbes were then inoculated and screened against the bacteria *S. epidermidis* and *Erwinia*. All four sites did not show any activity against *S. epidermidis*, while two of the four sites showed activity against *Erwinia*. The two groups that showed activity against *Erwinia* were selected and the two sites that did not show any activity randomly selected bacteria to screen against the ESKAPE pathogens. The agents from Garden number four showed activity against *E. coli* and *Acinetobacter*. Our results did not confirm our hypothesis as we thought all four sample sites would produce 2-4 antibiotic-producing agents and instead only one site produced 2-4 agents. Our research of the garden producing antibiotics from the four Northeast Wisconsin garden locations is still ongoing, therefore some results are to be determined.

6 Antibiotic Presence in Soil Samples from Rural and Urban Woodland Areas

KORINA HENNINGSEN, CHARLOTTE SKALA
Northeast Wisconsin Technical College

The growing prevalence of antibiotic-resistant bacteria and lack of new antibiotics to address them is resulting in higher mortality from formerly treatable infections. The Tiny Earth project aims to find novel antibiotics from microbes in soil samples collected and processed by college students. We collected soil samples from a woodland area in Green Valley and the woods of Bay View Park in Milwaukee. We wanted to compare a rural woodland sample to one from an urban park near Lake Michigan. We hypothesized that we would find one antibiotic-producing microbe from the woodland sample and one from the Milwaukee sample. After collecting our soil samples and diluting them, we plated the bacteria. We selected 20 colonies from each sample to inoculate and screen against the bacteria *Staphylococcus epidermidis* and *Erwinia* carotova to see which microbes might produce antibiotics against these bacteria. Any microbes that showed antibiotic activity against *S. epidermidis* or *E. caratova* were then tested against the eight ESKAPE pathogens. Both samples contained one bacteria that showed antibiotic activity against *S. epidermidis* in the initial 24 hours. When screened against the ESKAPE pathogens, the woodland bacteria showed antibiotic activity against *Escherichia coli* and *Acinetobacter baylyi* suggesting broad-spectrum activity. The Milwaukee sample showed activity against *A. baylyi* after the initial 24 hours. Our hypotheses about finding one antibiotic-producing microbe from each sample were correct. Our project is still ongoing, with additional results to be determined.

7 Antibiotics in the Soil

EMILY ABTS
Northeast Wisconsin Technical College

Recently, scientists have been struggling with antibiotic resistant pathogens. It is becoming increasingly difficult to find new antibiotics that can fight off these pathogens, leading to an increase in death rate. In an effort to find a new antibiotic, I took a soil sample from a swampy area in my backyard with the idea that the extra moisture in the soil and the cooler soil temperature would harbor unique bacteria. Early on, I hypothesized my sample would contain 10 antibiotic producing bacteria. I diluted my soil sample to isolate bacteria and cultured 20 different bacteria from the soil sample. I then took samples of my bacteria and put it on two different culture plates, one with *Erwinia* and one with *S. epidermidis*, to see if my bacteria produced antibiotics against them. Finally, I took my most promising bacteria colony and tested it on an ESKAPE culture plate. Although my bacteria proved to be effective against *Erwinia*, it did not produce more clearings on the ESKAPE plate. My original hypothesis that I would have 10 antibiotic producing bacteria proved to be incorrect as I produced one bacteria effective against *Erwinia*, one bacteria effective against *S. epidermidis*, further testing on the isolate will reveal its identity.

8 Beans or Corn? Which is the Better Antibiotic Producer

OLIVIA WILDER, LAUREN DEGRAVE
Northeast Wisconsin Technical College

Over the course of many decades' researchers have been perplexed with the evolving number of antibiotic-resistant pathogens, and the scarce resources to discover new antibiotics. Thus, causing an increase in mortality amongst the human population. The Tiny Earth project allows for discovery of new antibiotics, through the experimentation of soil in our preferred communities in attempts to find new antibiotic producing microbes. To relate to the community, soil samples were taken from a bean field and a corn field. Our hypothesis stated that the bean field in Shawano County would produce more antibiotic producing microbes than the corn field in Brown County, due to the fact that corn removes more nutrients out of the soil to grow. We removed our soil samples from our desired fields. Subsequently, we diluted and plated our soil from our respective fields. Afterwards, twenty different colonies were selected and placed on a master, *S. epidermidis*, and *Erwinia* plates. The bean field in Shawano County showed four possible antibiotic releasing microbes, and the corn field in Brown County showed one double producing antibiotic releasing microbe. After we discovered our possible antibiotic releasing microbes, we each selected one producer to assess them against ESKAPE pathogens. Our results concluded that the bean field produced against *Bacillus* and *Lysobacter*, whereas the corn field did not produce against any additional ESKAPE pathogens. Thus, our hypothesis was accurate. Our research is still ongoing, and further results are to be determined.

9 Antibiotic Producing Bacteria Found at the Dump Site in Woodside

ELISA HALL
Northeast Wisconsin Technical College

Due to the antibiotic crisis that we are facing today, Tiny Earth students are given a chance to become a scientist to research soil in our community to discover new sources of microbes that produce antibiotics. I chose Woodside Lutheran Homes, particularly the vicinity of the dump area. This is where all the garbage accumulated daily before being collected weekly. As a healthcare worker at Woodside, I noticed that some patients have wounds that must be changed daily, and we also have some cases of infections that happen occasionally. So, I'm hoping to discover one or two new microbes that produce antibiotic properties in that area. We performed different methods and procedures of cultivating bacteria from our soil sample. First, we diluted and plated our soil sample to encourage growth of colonies in different media. The results were very promising, lots of different colonies & bacteria grew on my plates. During culturing and inoculation of microbes against the bacteria *Erwinia* and *S. epidermidis* I found some zones of clearing in #6 and #15, double-producer microbes. I then chose one antibiotic producer and screened them against the ESKAPE pathogens. The results were inspiring, I found an increase in activity in *Bacillus*, *Pseudomonas* and *E. coli*. The most prominent was the *Pseudomonas* because it has a clear zone around this bacterium. In conclusion, I noticed a trend in my bacteria antibiotic activity that it's a double producer and it works in all gram type, shape and grouping of bacteria.

10 Dam it!

LISA MAJEWSKI, MAYA SANDOVAL, EMILY SEIDL, ALICIA STEMWEDEL
Northeast Wisconsin Technical College

For many years, scientists have grappled with the challenge of antibiotic-resistant pathogens. Our project tackles this pressing issue by exploring local dams and bodies of water for potential antibiotic-producing bacteria found in soil. We obtained soil from the Manawa dam/ millpond, Fonferek Glen in Green Bay, Oconto Falls dam, and the runoff of a family pond in Suamico. We hypothesized that we would find one antibiotic-producing bacteria between our samples. We used the soil dilution method on soil from each location to make the bacteria much less concentrated, which allowed plenty of room to grow and made it easier to count. We changed the culture conditions to determine if different types of growth would come from our samples. The master and tester plate method allowed us to test different colony samples against *Erwinia* and *S. epidermidis* for antibiotic-producing activity. We tested our samples against the ESKAPE pathogens utilizing the All-Tester Plate and Streak Plate methods. The Manawa dam/millpond sample displayed 7 antibiotic producers. The Fonferek Glen sample displayed one antibiotic producer. Our sample from the Oconto Falls dam displayed 2 antibiotic producers. One within 24 hours and one that took a couple of days to react. Our sample from a family pond runoff showed two antibiotic producers as well. Our hypothesis was incorrect because we found 12 antibiotic-producing microbes. Further testing is necessary. Therefore, some results are to be determined.

11 Defeating Antibiotic Resistance

HATTIE HERRMANN, BROOKE HAGENOW, CHLOE LEWIS
Northeast Wisconisn Technical College

Due to antibiotic resistance, new antibiotics are no longer being created. This is a big problem because of the increased numbers of sick people. To address this, we are searching to find undiscovered bacteria.

Our hypothesis is that one out of our three locations will contain undiscovered bacteria. The methods we used were soil collection and dilution. After dilution, we let the bacteria grow and then changed conditions to try to increase the number of colonies on the plates. Then picking 20 isolated colonies, spreading Erwina and S.epidermidis, we transferred pieces of our colonies to the plates. Next, we picked one colony to focus on to continue testing on 8 other bacteria to see which ones it killed. Most recently, we used a PCR test to amplify the genetic code of our bacteria. Together we discovered that our hypothesis was supported, that one of the soil samples contained antibiotic activity.

12 Antibiotic Producers in my Own Backyard?

OLIVIA MILLER
Northeast Wisconsin Technical College

In recent decades, scientists have encountered many problems with pathogens becoming resistant to antibiotics. This has made infections and illness more difficult for providers to treat, as well as the mortality rates increasing significantly. The opportunity was presented by the Tiny Earth Organization, to collect a soil sample from our community to try and discover a new antibiotic producing microbe. When deciding where I wanted to collect my soil sample from, I was interested to see if there would be any antibiotic producing microbes in my own backyard. My hypothesis was that my soil sample would have at least one antibiotic producer due to damp and dark conditions, making it more likely for the microbes to reproduce. I started with diluting and plating my soil sample using the streak plate method. My sample was inoculated and then tested against *Erwinia* and *S. epidermidis* to see what microbes, if any, would produce antibiotics against the bacteria. I found that my sample had two antibiotic releasing microbes, one microbe was a dual producer against both *Erwinia* and *S. epidermidis*, while the other only produced against *S. epidermidis*. I then chose to move on the research with my dual producing microbe and test how it would react against the ESKAPE pathogens. The results showed that there were no antibiotics produced against those pathogens. My hypothesis was not accurate, since my soil sample had two antibiotic producing microbes instead of one. My research on antibiotic producing microbes from my own backyard is still on going, therefore results are to be determined.

13 Unearthing the Mysteries in Our Own Backyard

CHRISTOPHER CONRAD. NADIA JAVIER
Northeast Wisconsin Technical College

Over the last few decades, antibiotics have been slowly becoming less and less effective; fault can be attributed to incorrect prescriptions, inappropriate or incomplete usage, and/or the natural mutation of pathogens themselves. Regardless, there are now microbes stronger than our most potent antibiotics and it's too expensive to continually research and develop new ones. The Tiny Earth Project gave us the opportunity and resources to find new antibiotics in our community which could help address this problem. Our group took two adjacent soils samples found in a family's backyard. One sample had luscious green grass growing from it, the other was absent of plant life. Our hypothesis is that the sample without plant life could have unique microbes that prevent the normal biota and plant life from growing. This would mean that these microbes could be antimicrobial and are active against other bacteria. To test this, we took those samples into lab and created soil dilutions to obtain distinguishable bacterial colonies. We then each tested twenty independent colonies against weaker bacteria that were different in Gram type, shape, and arrangement (*Erwinia* and *Staphylococcus epidermidis*). Based on those results, there was no activity on the sample with grass therefore we selected two colonies from the lack-of-plant-life sample that showed the most antibiotic activity and placed them on a tester plate with eight additional bacterial species to further study our colony's antibiotic properties. Our results showed both did well against nearly all the tester bacterial species. Depending on the results of the DNA sequencing, it's possible we have one or two unique strains of bacteria that are active against other bacteria.

14 Could a Novel Anti-B Await Discovery In Your Backyard, Under a Rock or Under a Tree?

KAILAM CARWIN, JANA HAMACHEK, AALIYAH BOTTORFF
Northeast Wisconsin Technical College

As our current antibiotic climate is slow growing in new discoveries and greater in growing resistance to current anti-b's – an urgent need has presented in search of new antibiotics. As once curable illnesses have turned untreatable and as new pathogens emerge, preventable deaths are mounting and a race against time has begun with continued searches underway. As such, we are partnering with the Tiny Earth Organization to share our findings with the scientific communities. Our team has collected soil samples in Wisconsin from under a tree at a local Botanical Garden, beneath a plant in a home backyard and under a rock at High Cliff State Park. We hypothesized our rock and tree samples would yield one microorganism that was an antibiotic producer and that our backyard sample would contain four. Upon further testing of soil collections by dilution and plating using a streak plate method, we mimicked the natural environmental conditions and grew our microbes. We proceeded to inoculate and screen them against the bacteria S. epidermidis and Erwinia, to detect possible antibiotic producers. Our Botanical and State Park samples each contained one possible antibiotic releasing microbe, whereas our backyard sample contained two, and all of which produced clearing against S. epidermidis. After selecting one microbe from each plate to test against ESKAPE pathogens, our results concluded that our backyard soil sample generated activity against two additional pathogens, whereas our rock and tree samples had no additional activity. Our hypotheses were disproven, but our research continues with additional testing.

15 Playing With Dirt

HOPE KOSMERCHOCK, STEPHANIE SCHNEIDER
Northeast Wisconsin Technical College

We need new antibiotics to treat bacterial infections that resist current medicines. The Tiny Earth Project aims to find new bacteria in soil that produce antibodies. We collected soil samples from two backyards: one from an older yard and another from a newly seeded yard. We expected the older yard to have more antibiotic-producing microbes due to more mature soil. Using the streak plate technique, we diluted and plated the samples, checking the microbes for antibiotic activity against *Staphylococcus epidermidis* and *Erwinia* for antibiotic production. We also tested one microbe from each sample against relatives of ESKAPE pathogens, which are resistant to multiple drugs. Our results showed that neither sample had antibiotic-producing microbes, which was unexpected. We plan to use PCR testing to confirm the bacterial strains and explore their potential for producing antibiotics. Our research is ongoing, and we anticipate more results soon. We hope our findings will help in the search for new antibiotic options to address antibiotic resistance in medicine today.

16 Soil Collection from Baird Creek Trail for Antibiotic Production Research

AMINA MOHAMED
Northeast Wisconsin Technical College

Over the last several years, the powerful strength of antibiotic resistance has raised a huge concern leading to deep analysis and research by scientists. Such concerns arose when antibiotic resistance became a reason for amplified bacterial growth and disease occurrence causing higher death rates. Through the Tiny Earth organization, I and other students from NWTC were granted the chance to collect soil from chosen areas of the community to research and possibly locate microbes which produce antibiotics. I made the decision to gather my soil from a nearby public trail at Baird Creek on the East side of Green Bay. My hypothesis was that: picking out soil from a more "damper" section at the trail closer to water (from East River watershed) area will include microbes with effective antibiotics because an environment with water can be crucial for bacterial growth. When the soil sample was brought to the lab, the process began by diluting soil and plating soil via a streak plate. Then, the produced microbes got inoculated, incubated (in chosen environment/condition, e.g., R2A dark), and tested against *Erwinia* and *Staphylococcus epidermidis* (*S. epidermidis*) to indicate the microbes that were able to make antibiotics against each. S.epidermidis showed an activity/antibiotic effect. Afterwards, choosing the antibiotic producer, it was screened ESKAPE pathogens, which displayed that there was no further bacteria antibiotic activity. My research of Baird Creek antibiotic producing soil is in progress and other results/conclusions are to be completed.

17 Barkteria

HAYLEY JOHNSON, ALAYNA FEIDT
Northeast Wisconsin Technical College

Between 1998 and 2002 there have been no new antibiotics found to help with bacterial infections, causing the antibiotic crisis according to the Tiny Earth research guide. This is dangerous because some bacteria have become resistant to all current antibiotics from overuse and over prescription. In Microbiology we were introduced to the Tiny Earth Project, which allows students to learn about this crisis, and help find new antibiotic-producing bacteria and inform others of this problem. We were instructed to collect soil samples from various places and compare the environments where they were taken from and how bacteria can grow. Both of us collected samples from the Clintonville and Kaukauna dog parks. Both samples were from dry, sunny areas but different texture soils. The Kaukauna sample's texture of the soil was grassy, and the Clintonville sample soil was sandy. The hypothesis for the Clintonville sample was that no antibiotic producing bacteria would be found. The hypothesis for the Kaukauna sample was that two antibiotic producing bacteria would be found. In class, we performed various tests against microbes to see if any of them would react against the bacteria collected at the dog parks. The Clintonville sample had some activity against *Acinetobacter* and *Bacillus* whereas the Kaukauna sample did not have any activity against any of the tester plate microbes. After the results we received from our bacteria we have continued ongoing tests against both samples.

18 Tiny Earth Project

HLEE XIONG, KARA LAMBRECHT
Northeast Wisconsin Technical College

No new antibiotic-producing bacteria have been found since the 1990s. Due to no new discoveries of antibiotic-producing bacteria, there is a nationwide search for new discoveries. The purpose of the Tiny Earth Project is to find antibiotic-producing bacteria to help prevent antibiotic-resistant from spreading. The soil collected from Red Arrow Park in Manitowoc, WI, was hypothesized not to have any antibiotic-producing bacteria. While, the soil that was collected from Doyle Park in Little Chute, WI, was hypothesized to have very little antibiotic-producing bacteria. Throughout our lab classes, we have used many methods to discover antibiotic-producing bacteria in our soil. We diluted the soil that we collected from each park to get the most isolated colonies. Many culture conditions were tried, including incubating in the dark and the R2A plate. We tested different colonies of our bacteria against several relatives of the ESKAPE pathogens. The bacteria from Red Arrow Park showed weak activity against *Bacillus*. Compared to the bacteria from Doyle Park, there was no activity against the pathogens tested. We used PCR to identify the 16s rRNA gene in our bacteria. The bacteria that we have experimented with so far have shown weak to no response against pathogens, but our experiments are still ongoing.

19 Antibiotics from the Earth: Searching Soil for Nature's Cure

YASMIN MOHAMED, VONGAI HOVE
Northeast Wisconsin Technical College

The Tiny Earth project assists in exploring the solutions for fighting antibiotic-resistant pathogens by researching potential antibiotic-producing bacteria from soil, which has been a challenge for quite some time now. Integrating this hands-on research advances the understanding of antibiotic resistance and new microbes that could produce antibiotics for students and the community. With the latest discovery of a new antibiotic class reaching the market in 1987, we know how important it is to find new and promising modern innovations in this field. To help start this discovery, we collected our soil samples from two different spots alongside the creek at Pamperin Park. The locations were both moist environments. The goal was to observe the similarities in the microbes given that the soil was exposed to the same naturally occurring environment with moisture, grass, rocks, animals, and stream water. Our hypothesis for both samples was to get at least two antibiotic-producing bacteria. The first step of this experiment started with soil dilution from which we obtained the desired solution concentration. The soil sample from the pathway resulted in some activity against the *S. epidermidis* bacteria compared to the soil sample gathered from the stream, which showed little to no activity. These colonies from the soil dilution shared a similar color, shape, and pattern. We modified the media conditions to change the type of growth of our bacteria by using Water R2 agar and 37 degrees Celsius incubation for both samples. Our research on soil microbes is still ongoing..

20 Discovering Antibiotic Producing Bacteria in Soil

ASHLEY CHARAPATA
Northeast Wisconsin Technical College

Over the last century, bacteria has started becoming resistant to antibiotics which is a global problem that scientists are working to solve. Misusing and overusing antibiotics has led to bacteria becoming resistant. Pathogens become resistant to antibiotics and scientists are constantly trying to find new antibiotics to use. Infections that are antibiotic-resistant can develop into lengthened infections and death. These infections can become untreatable by any already existing antibiotics and can only be cured with finding new antibiotics and this becomes an endless cycle of antibiotics becoming useless. The Tiny Earth organization has given our microbiology class the opportunity to research soil from locations near us and to deliver our results at a conference. I took my soil sample from the grassy backyard of my house about an hour north of Green Bay. My hypothesis was that I would find zero to one antibiotic producing bacteria in my soil sample because I understand that finding these is rare. I used the soil dilution technique and spread the diluted soil onto streak plates. I then grew my soil dilution on plates with two different types of media. I then tested my soil on an *Erwinia* plate and an *S. epidermidis* plate. I chose one of my isolated colonies to do further testing. I tested my colony on a master plate with eight ESKAPE pathogens. My bacteria worked against four types of ESKAPE pathogens.

21 The Effect of Lake Proximity on Antibiotic Production in Soil Bacteria

ELIZABETH YRAY, ELLERY HANSEN, ALONDRA CHAVEZ AND LAUREN HOLTHAUS
University of Wisconsin - Madison

Our study investigates how the proximity of soil samples to Lake Mendota, the center of the UW-Madison campus, influences their microbiome's antibiotic production properties. We hypothesize that soil closer to the lake exhibits stronger antibiotic production properties due to interactions between lake and soil microbiomes, with using the zone of inhibition as our indicator. The soil samples were selected from three distances from the lake—0.5 meters into the lake, 0.5 meters away from the lake, and 10 meters from the lake. Master plates were created using these colonies using dilutions catered to single-colony selection and zones of inhibitions were recorded when screened with ESKAPE relatives. The difference in antibiotic-producers per group were analyzed using a Fisher 2x3 test and we failed to reject the null hypothesis with a p-value of $p=0.31$. Although the null hypothesis was not rejected, this study asks questions and opens doors to broader questions of environmental effects in this rising global health crisis.

22 Antibiotic Resistance in Soil; Comparing Golf Courses and Backyard Bacteria

KAITLYN RICH-FALK, TAYAH CONRAD, LIZZIE WILKINS
Northeast Wisconsin Technical College

In recent decades, antibiotic resistance has emerged as a significant global health concern, contributing to increased mortality rates worldwide. The Tiny Earth Project provides an opportunity for college students to explore potential new antibiotics, allowing them to present their findings to the scientific and medical community. Throughout the semester, we collected soil samples from three locations: two golf courses and a backyard. Our hypothesis stated that the backyard soil would yield more antibiotic activity due to its exposure to a wider variety of organic matter, including animal feces and chemical substances like fertilizers. To identify new antibiotic properties, we employed a systematic approach. We diluted and plated our soil samples using a streak plate technique, then inoculated the isolated microbes for screening against various bacteria. Unexpectedly, the backyard and second golf course samples exhibited no antibiotic activity against any tested bacteria. In contrast, the first golf course sample demonstrated resistance against *Staphylococcus epidermidis*. Thus, our initial hypothesis was incorrect, as only the first golf course sample produced any antibiotic resistance. Ongoing research at NWTC will further investigate these findings, with some results still pending.

23 The Race against Antibiotic Resistance

HALLE GUSTAFSON
Northeast Wisconsin Technical College

Antibiotic resistance is becoming a wide spreading issue in today's world. Finding novel antibiotics is important in helping mitigate some of the problems we are facing due to antibiotic resistance. Research on novel antibiotics is currently being student sourced through the Tiny Earth research program. I collected a soil sample from my own land, which I suspected to be a suitable environment for microbial growth due to the high level of moisture in the soil. I was confident in the amount of antibiotic producers I would find as I hypothesized finding seven producers in my sample. I diluted my sample and utilized streak plating to grow the sample colonies. From there I tested my sample colonies against *Staphylococcus epidermidis* and *Erwinia carotovora* in order to analyze any antibiotic production that may be present. I had four colonies that showed signs of antibiotic production. I moved forward with the one colony that was a dual producer against both *S. epidermidis* and E. carotovora, and tested that isolate against the ESKAPE pathogens. The results showed three zones of inhibition, demonstrating antibiotic production. My hypothesis was inaccurate as I estimated finding seven producers, of which I only found three in my sample. The novelty of my antibiotic producers is yet to be determined, as research is still underway.

24 Soil Superstars: Discovering Antibiotic-Producers Right Under Our Feet

SOFIA ORELLANA, CALLIE HUBATCH
University of Wisconsin - Green Bay

Soil is a rich and diverse ecosystem teeming with microorganisms, among which are bacteria capable of producing antibiotics. These antibiotics-producing bacteria, particularly from genera such *Streptomyces*, *Bacillus*, and *Pseudomonas*, are critical in natural competition, using antibiotics to inhibit or kill competing microorganisms. Antibiotic resistance is a significant issue today, and the shortage of effective antibiotics poses a growing threat to society. The natural production has significant implications for human medicine. Soil bacteria have historically been a major source of antibiotic discovery including drugs like streptomycin, tetracycline, and erythromycin. The purpose of this research was to identify soil bacteria capable of producing antibiotics. The methods used in this study focused on identifying antibiotic-producing bacteria which included an isolate screening using *Escherichia coli (E. coli)* and *Bacillus subtillis* (*B. subtillis*) to determine the antibiotic production. Through PCR and BLAST analysis, we identified *Pseudomonas* and *Acinetobacter calcoaceticus* as antibiotic producers. Advances in molecular biology and genomics are now facilitating the study of these organisms, enabling scientists to understand the biosynthetic pathways responsible for antibiotic production, which could lead to synthetic modification and development of more effective drugs.

25 What's Lurking in our Waters?

JENNIFER ROSS, ELLA JUNION AND BRIANNA GEIGER
Northeast Wisconsin Technical College

Over the last semester, we have set out to try and discover bacteria that produces antibiotic material. We are taking part in the Tiny Earth Project in efforts to combat antibiotic resistance that is happening all over the world today. We became part of the Tiny Earth Project to see if antibiotic-producing bacteria exist in the soil surrounding bodies of water in Brown and Outagamie counties, experiments are ongoing on each of the bacterial samples. One student chose her soil from a man-made pond in De Pere. Another student chose to get her soil sample from the lake at High Cliff State Park. The third student chose her soil from the pond at the Kaukauna dog park. We predicted we would find one antibiotic-producing bacteria because of the abundant amount of microorganisms. From our samples we collected, we completed each test of soil dilution, culture conditions and finally, tested for antibiotic activity against relatives of the ESKAPE pathogens. The "all tester" streak plates that we tested each sample with were *S. epidermidis*, *E. coli*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Bacillus*, *Mycobacterium* and *Lysobacter*. With our current results from this streak plate, our findings were inconclusive. Our group will continue to keep testing our bacteria or identify our unknown bacterial samples until we find an antibiotic-producing microbe or an unknown microbe.

26 Comparative Analysis of Soil Properties from Two Urban Parks: Hoping to End Antibiotic Resistance Crisis

BRITTANY KURTZ MAROTZ, JILLIAN JOSEPH
Northeast Wisconsin Technical College

Antibiotic resistance is a growing global health concern that poses significant challenges to the treatment of infectious diseases. We are hoping to discover types of antibiotics in hopes to isolate a new type and to diminish the growing rate of antibiotic resistance we are facing. We obtained dirt from two different parks, one in De Pere and the other on the east side of Green Bay, in order to find different but compatible data. We predicted that De Pere would have three antibiotic producing bacteria and Green Bay would have zero. Once we obtained our dirt it went through many processes of dilution and isolation. Then we chose 20 colonies from each sample to test against *Erwinia* and *S. epidermidis*. Both of our samples showed activity against *Erwinia* and *S. epidermidis*, but we did not have any repeating on both. Neither of our two samples showed additional activity against ESKAPE pathogens. Our hypothesis was incorrect as there were two producers found in each sample. Our findings, through PCR and Gel Electrophoresis testing, showed that one sample was positive, and one was negative. The one sample that was positive came from the park in Green Bay and was sent to UW Madison for sequence analysis. Experiments are ongoing, and more data is to be acquired.

27 Antibiotic Production in Soil Bacterial Colonies Treated with Differing Concentrations of Colloidal Silver

ALINAH LIU, AVA PREBLE, SAUL PRASKA
University of Wisconsin - Madison

There remains a pressing need for further antibiotic development research as the antibiotic-resistance crisis worsens. One hurdle in the pursuit of bacteria derived antibiotic discovery in a laboratory setting is unculturability in standard growth conditions. By introducing a stressor, common bacteria that usually appear from soil samples may be outcompeted by previously unknown bacteria. Silver has been the subject of antimicrobial research and its relationship to antibiotic resistance. By breaking down bacterial membranes, it provides a harsher environment for bacteria and could promote growth of previously uncultured bacteria. We measured the effects on growth of antibiotic-producing bacteria against ESKAPE relatives with different dilutions of colloidal silver in the growth media. The results gathered from plates did not show a significant difference between silver treatments and our control, however, several antibiotic-producing colonies were found and further analyzed.

STUDENT POSTERS

28 The Effect of Apple Cider Vinegar Treatments on the Growth of Potential Antibiotic-Producing Soil Bacteria

MAHA MEGHANI, ALLISON BRADLEY, CARSON BEACH, REESE FRIGHETTO
University of Wisconsin - Madison

In response to the antibiotic resistance crisis, apple cider vinegar (ACV) may be a plausible mechanism to stimulate antibiotic production in soil bacteria. We grew soil bacteria in three concentrations of ACV and tested the resulting colonies against ESKAPE relative pathogens to evaluate antibiotic producing capabilities. Although ACV is an effective antimicrobial, it does not seem to promote the growth of antibiotic producing bacteria. When analyzing data, we found that 62 or 27% of the soil bacteria colonies were antibiotic producers to at least one ESKAPE pathogen. Our findings suggest that ACV can function as an antimicrobial agent on its own but did not significantly enhance antibiotic production in the soil bacteria populations of interest.

29 Effects of Maple Syrup in Agar Plate Media on Bacterial Growth and Antibiotic Properties of Soil Bacteria

KATE CLARK, THI TRUONG
University of Wisconsin - Madison

The antibiotic resistance crisis is an ever-growing issue that threatens millions of lives, leading to the need to discover new antibiotics. Growth medium was altered to test the effects of varying concentrations of maple syrup on bacterial growth. Bacterial colonies from soil samples were plated and exposed to various relatives of known pathogens, zones of inhibition were quantified, and PCR was performed to classify antibiotic-producing bacteria. Preliminary results show that there is no statistically significant difference between concentration of maple syrup and proportion of antibiotic-producing bacterial colonies. Although there was no statistically significant impact of the maple syrup treatment, future research should explore adding different volumes of syrup or other antioxidants to the growth medium.

30 Whatttttt: Antibiotics in my backyard!!

KAYLA PEREZ
Northeast Wisconsin Technical College

Have you ever heard of antibiotic resistance? Well, it has been quite an issue for some time, mainly because of the human population. Antibiotic resistance happens when antibiotics are taken for something other than bacterial infection, treatment is not fully completed, or antibiotics being overused. All the factors can contribute, meaning the bacteria multiply and become harder to fight and our current medications become ineffective. There have not been any new antibiotics released in decades, but that does not mean scientists are not hard at work. The Tiny Earth Project is a research-based project for many college students to help scientists find new bacteria that live in soil in hopes of discovering new antibiotic treatments. To help with this, and keep things simple, I of course collected my sample from my own yard in Green Bay, WI. Coordinates 44`31'22" N 88`01'15" W, temperature was sunny/sunset 75 at 6:48 pm with wind gusts up to 12 MPH. My Hypothesis was to get at least 1 antibiotic producing bacteria, in which my hypothesis was correct with 1. Upon testing my sample showed no activity against *S. epidermidis* Gram positive or *Erwinia* Gram Negative. I then decided to just pick a sample off my master plate for further testing against ESKAPE pathogens. Results showed positive against *Acinetobacter baumanni* gram negative ESKAPE pathogen. Currently, further testing is being conducted regarding gel electrophoresis in hopes of helping the Tiny Earth project.

31 Novel Antibiotics Unearthed Continued

THAO DESAHNI
Green Bay West High School

Tiny Earth research addresses the global crisis of the decreasing number of useful antibiotics. To expand my original Tiny Earth research project from last year, I decided to focus on two unique sources of antibiotic-producing bacteria. My soil specimen was taken from the Brussels Hill Pit Cave in Door County, WI in 1987. My second sample was sediment shavings from rocks from around the world. A soil sample was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate soil bacteria. We screened isolated bacteria for antibiotic activity against *E. coli* and B. subtilis on LBA, potato dextrose agar (PDA), 100% tryptic soy agar (TSA), 10% TSA plates. I was able to identify antibiotic activity with isolates 1-4, and 9. Isolates 1-3, and 9 display narrow-spectrum antibiotic activity, while isolate 4 display broad-spectrum antibiotic activity against E.coli and *B.subtilis*. We gram stained the soil isolates to identify the gram reaction and morphology of the soil isolates. Through the Colony PCR method and BLAST analysis we determined our soil antibiotic identity. To ensure that the identity of our soil isolates aligned with our gram stain and BLAST analysis, we conducted a series of biochemical tests. This extended research project explored two unique sources of antibiotic-producing bacteria. Both samples contained bacteria with antibiotic activity against *Bacillus subtilis*. An isolate from the rock shavings displayed broad spectrum antibiotic activity. These data suggest that unique sample sites may be valuable sources of antibiotic-producing bacteria.

32 A Prairie of Possibilities Under Our Feet

ISABELLA WERY-TREJO
Green Bay West High School

The increasing resistance of existing antibiotics, paired with the lack of new antibiotics, is a significant threat to global health. Tiny Earth is a research project to study antibiotics produced by soil bacteria to aid the global antibiotic crisis. Soil was collected on the UW-Green Bay campus (44.52809 N 87.92649 W). One gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate and select for soil bacteria and not fungi. Isolated colonies were picked and patched to LBA and potato dextrose agar (PDA) to create library plates for additional experimentation. Antibiotic activity was screened by placing isolates on diverse media, including LBA, PDA, tryptic soy agar (TSA), and 10% TSA containing either *Bacillus subtilis* (B. subtilis) or *Escherichia coli (E. coli)*. Six isolates out of 12 isolates displayed antibiotic activity. Antibiotic producers were gram stained. BLAST analysis of the DNA sequence for the 16S rRNA gene, along with several biochemical tests were conducted to identify the genus of each antibiotic producer.

33 Antibiotic Activity Beneath Our Feet

MONSERRATH SANCHEZ
Green Bay West High School

There is a diminishing supply of effective antibiotics to treat bacterial infections. Tiny Earth research focuses on studying antibiotic activity in soil bacteria because soil contains a diversity of antibiotic-producing bacteria. A soil sample was collected from the UW-Green Bay Coffin Arboretum (44.52878°N, 87.92281°W) because of the great deal of vegetation in the site. One gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) plates with cycloheximide. Isolated colonies were patched to LBA and potato-dextrose agar (PDA) plates to create library plates. Isolates were screened for antibiotic activity against *B.subtilis* and *E. coli* on a variety of media including, LBA, PDA, 100% tryptic soy agar (TSA), and 10% TSA plates. Isolate #2 and #3 displayed antibiotic activity against *B.subtilis* on PDA, 100% TSA, and 10% TSA media. Isolate #3 was identified as gram-positive, streptococci-shaped bacteria. Isolate #2 was identified as gram-positive, rod-shaped bacteria. Polymerase Chain Reaction (PCR) was performed to amplify the DNA sequence for the 16S rRNA gene to identify each isolate. Isolate #2 belongs to the genus, *Bacillus*, agreeing with Gram-stain analysis. Isolate #3 could not be identified in this study. Isolate #2 was subjected to several biochemical tests to confirm BLAST data. Tiny Earth research is valuable because it is a way to find new antibiotics from soil, a readily accessible resource.

STUDENT POSTERS

34 The Earth's Medicine Cabinet: Discovering Antibiotics in Soil

CECELIA PIERQUET
Green Bay West High School

Tiny Earth addresses the health crisis of antibiotic resistance by studying soil samples to find antibiotic-producing bacteria. A soil sample was collected at UW Green Bay campus (44.52724°N, 87.92548°W). One gram of soil was serially diluted and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate individual bacteria. Six isolates were patched to Potato Dextrose (PDA) and LBA to create library plates. To screen for antibiotic activity, isolated bacteria were patched from library plates to 10% Trypticase Soy agar (TSA), TSA, LBA, and Potato Dextrose agar (PDA) containing either *Bacillus subtilis (B.subtilis)* or *Escherichia coli (E. coli)*. Isolate #3 showed antibiotic activity against B. subtilis on 10% TSA, TSA, and PDA. Isolate #4 displayed antibiotic activity against B. subtilis on 10% TSA and LBA plates. Isolate #5 displayed antibiotic activity against *E. coli* and B. subtilis on 10% TSA, PDA and *B.subtilis* on LBA plates. By gram staining, isolate #3 is gram-positive, rod-shaped bacterium. Isolate #4 is gram positive and isolate #5 is gram negative. Isolate #3 belongs to the genus, *Paenibacillus*, according to BLAST analysis of the DNA sequence of the 16S rRNA gene. This research is valuable because it can help facilitate progress toward finding new antibiotics to help with the world crisis of antibiotic resistance.

35 Soil Secrets: The Hidden World of Natural Antibiotics

ADALYN SIHARAJ
Green Bay West High School

Tiny Earth research addresses the global health crisis of antibiotic-resistant bacteria by studying antibiotic activity of soil bacteria. A soil sample was collected from the UW-Green Bay Campus (44.52746°N, 87.92512°W), serially diluted in sterile water, and placed onto Luria-Bertani agar (LBA) plates containing cycloheximide. Isolated bacteria were patched to LBA and potato dextrose agar (PDA) plates to create library plates. Isolates were screened for antibiotic activity by patching them to Tryptic Soy agar (TSA), 10%TSA, PDA, LBA containing *E. coli* or B. subtilis. One of six isolates displayed antibiotic activity against B. subtilis on PDA and 10%TSA. This antibiotic producer (i.e., isolate #3) was determined to be a gram-positive, rod-shaped bacterium by gram stain analysis. Isolate 3 belongs to the genus, *Bacillus*, according to BLAST analysis of the 16S rRNA gene and confirmed by gram stain analysis and several biochemical tests. Future implications, this research will help to minimize antibiotic resistance through the discovery of new antibiotics, which will in turn help to combat the overarching global health crisis.

36 Digging for a Cure: The Hunt for Soil-Based Antibiotics

MARISOL MAGANA
Green Bay West High School

Antibiotic resistance is a global health crisis. Tiny Earth antibiotic discovery research has moved us closer to solving this global health crisis. This research project began with a soil sample that was collected from Green Bay, West High School (22.52197 N, 88.03411 W). One gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) with cycloheximide to select for soil bacteria. Isolated colonies were patched to LBA and potato dextrose agar (PDA) to create library plates for additional experimentation. Soil isolates were screened for antibiotic activity by patching these bacteria on LBA, PDA, Tryptic Soy Agar (TSA), 10% TSA, and Potato Dextrose Agar containing either *Escherichia coli (E. coli)* or *Bacillus subtilis* (B. subtilis) as test strains. A soil isolate showed antibiotic activity against B. subtilis on TSA, as evidenced by a zone of inhibition. Gram stain analysis, several biochemical tests and polymerase chain reaction (PCR) were conducted to characterize the identity of the antibiotic-producing soil isolate. PCR amplification of the DNA sequence for the 16S rRNA gene was attempted, but inconclusive. Upon gram staining, the antibiotic-producing isolate was identified as a gram-positive, rod-shaped bacterium. Additional studies to identify the chemical structure of the compounds inhibiting B. subtilis on LBA are required to explore further the clinical implications of the experimental findings reported here.

37 Journey to: Discovering Antibiotics

ELIZABETH VERA
Green Bay West High School

With the increase of antibiotic resistance surging throughout the world, it is time for the advancement and discovery of new and improved antibiotics to ensure we have a healthy future. To achieve this, a soil sample was collected at the bottom of a tree by a pond in the UW-Green Bay Cofrin Arboretum at (44.53719° North, 87.92546° West). One gram of soil was serially diluted in water and plated onto Luria-bertani agar (LBA) and potato dextrose agar (PDA) to create library plates. To screen for antibiotic activity, individual isolates were patched to tryptic soy agar (TSA), 10% TSA, PDA, and LBA plates containing either B. subtilis or *E. coli* test strains. Isolate #1 showed antibiotic activity against E.coli on the LBA plate. Through the Gram stain analysis, the antibiotic producer was identified as a gram-positive, rod-shaped bacterium. The isolate was subjected to 8 biochemical tests to characterize the traits of the organism. This project proves to be valuable because it is designed to discover antibiotics for the benefit of the world.

38 One Sample a Million Possibilities

NAZARETH YANEZ
Green Bay West High School

Over the last 30 years, 30 million people have died from infections caused by antibiotic- resistant bacteria. Tiny Earth focuses on searching for new antibiotics in order to address this global crisis. The research project began with a soil sample collected on the UW- Green Bay campus near a wooded area (44.52689°N, 87.92603°W). One gram of soil was serially diluted in water and plated on a Luria-Bertani agar (LBA) plates containing cycloheximide in order to separate bacteria from one another. Isolated bacteria were patched to PDA and LBA agar to create library plates, creating a surplus of individual bacteria for additional studies. Soil isolates were screened for antibiotic activity by patching isolates on PDA, LBA, 10% tryptic soy agar (TSA), and 100% TSA plates containing *E. coli* or B. subtilis. Four isolates (3,4,5 and 8) displayed antibiotic activity. The isolates were gram stained, identified by BLAST analysis of the DNA sequence for the 16S rRNA gene. Isolates 8, 3, and 5 were identified as *Pseudomonas*, and two species of *Bacillus*, respectively.

39 The Golden Age of Antibiotic Discovery

DORCAS BIGIRIMANA
Green Bay West High School

The need for new antibiotics is a growing global health crisis. Tiny Earth research helps to address the public health crisis by investigating bacteria found in soil to search for new antibiotics. The present research focuses on bacteria found in soil due to the rich diversity and unique bacteria present there. A gram of soil was collected at UW-Green Bay on the Arboretum trails (44.52776 N, -87.92756 W). The soil sample was serially diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide to select for prokaryotes and make it less concentrated for further studies. Using the “pick and patch” technique, isolates of interest were transferred onto LBA to create master plates. Antibiotic activity of the isolates were screened against *Bacillus subtilis* and *Escherichia coli* on LBA, Potato Dextrose Agar (PDA), 100% Tryptic Soy agar (TSA) and 10% TSA. Isolate 4 exhibited antibiotic activity against *B.subtilis* on LB. Isolate 4 grew and spread on *B.subtilis*. We then used the polymerase chain reaction technique to make multiple copies of specific DNA regions used to identify the genus of the isolate. Through polymerase chain reaction, the genus of the isolate was determined using the Basic Local Alignment Search Tool (BLAST). Isolate 4 was determined to be *Pseudomonas*, which was verified through the gram-stain and biochemical tests. This research is important for developing new antibiotics, potentially helping individuals who would otherwise be without treatment options. Future implications are to send the Isolate to Tiny Earth.

STUDENT POSTERS

40 The Path to Antibiotic Discovery: Secrets in the Soil

ADRIANA FLORES TORRES
Green Bay West High School

The global antibiotic resistance crisis has become a prominent global health issue in today’s modern age where overconsumption of antibiotics makes the problem increasingly worse. The research in the Tiny Earth program aims to find a solution to this problem, researching soil with a rich medium containing diverse populations of antibiotic-producing bacteria. During this research, a soil sample was taken from the UW - Green Bay Cofrin Arboretum (44.52655°N, 87.92643°). One gram of soil was serially diluted in sterile H2O and plated onto Luria-Bertani agar (LBA) plates. Isolated colonies were picked and patched onto LBA and potato dextrose agar (PDA) to create library plates. Isolates were tested for antibiotic activity against *B.subtilis* and E.coli on different media. Out of nine isolates, #2, 3, 4, 7, 8, 9 showed antibiotic activity against *B.subtilis*. The 6 isolates were gram stained. The polymerase chain reaction (PCR) was performed on each isolate to amplify the 16S rRNA gene, in order for it to be sequenced. The sequences were then entered into the BLAST database to identify the genus of the isolates, which were identified as four species of *Bacillus* and two *Pseudomonas* isolates. Eight different biochemical tests were performed on the 6 isolates to observe their properties and reactions. This research is valuable because it leverages the diversity of bacteria in soil as a strategy to discover novel antibiotic-producing bacteria to address antibiotic resistance.

41 Surfing the Soil: How One Soil Sample Could Save Millions

DAYANA ZAMORA VAZQUEZ
Green Bay West High School

There is a growing need for new novel therapeutics due to the emergence of antibiotic resistance, making it significantly more difficult to treat illnesses that were previously treatable. The Tiny Earth research initiative seeks to combat this issue through the research into novel antibiotic producers found in soil. A soil sample was collected from the UW-Green Bay Campus (44.5267352 N, -87.9257653W). The soil sample was serially diluted in sterile water, plated on Luria-Bertani agar (LBA) plates containing cycloheximide and incubated at 28°C. The “pick-and-patch” technique was used to create library plates on LBA and Potato Dextrose agar (PDA). The “spread-patch” technique was utilized to screen for antibiotic activity against *Bacillus subtilis* (B. subtilis) and *Escherichia coli (E. coli)*. The samples were plated onto the LBA, PDA, 10% Tryptic Soy Acid (TSA), and TSA plates. Antibiotic activity against *E. coli* was identified for isolates 1 and 3, while antibiotic activity against *B. subtillis* was identified for isolates 3 and 5. Isolate 1 is the only broad-spectrum antibiotic-producing bacterium. After gram-staining, isolates 3 and 5 were identified as gram-negative rods. Isolate 1 was identified as gram-positive cocci. The polymerase chain reaction (PCR) was used to amplify the DNA sequence for the 16S rRNA gene, which was subjected to Basic Local Alignment Search Tool (BLAST) analysis to identify the genus of each isolate. These findings could provide a starting point into the further study of the behavior of antibiotic producers from diverse soil environments.

42 The Search for Antibiotics in Pamperin Park, Wisconsin

CHRISTIANNA LIEBERENZ, OLIVIA BRZOZOWSKI
Northeast Wisconsin Technical College

Antibiotics are used to treat bacterial infections by either killing them or preventing them from spreading. Unfortunately, bacteria are constantly changing and evolving their genetic material to resist existing antibiotics. Because of this, scientists are always on the search for new, effective antibiotics to continue fighting off these infections. The Tiny Earth Initiative allowed us to collect soil in our community and test it for microbes that release new antibiotics. We decided to collect our samples from Pamperin Park in Green Bay, Wisconsin. We chose Pamperin Park because it was easily accessible and because it is a large, diverse landscape. We decided to collect one of our soil samples from a wet environment inside the creek, and the other from a dry environment away from the creek in the hopes of comparing our findings. We predicted that the wet environment would produce more antibiotic microbes than the dry environment because microbes reproduce more under wet conditions. We started by diluting both our soil samples and spreading them on a streak plate to get isolated colonies. Then, we each tested 20 colonies from our sample plate for antibiotic activity. We found that we had one antibiotic releasing microbe from the dry environment, which was *Bacillus*, and none from the wet environment. This means our hypothesis was false and the dry environment produced more antibiotic microbes than the wet environment. Our experiment is still ongoing, so some of our results still need to be determined.

43 Unearthing Natures Medicine

ADRIANA MOYANO
Green Bay West High School

With the emergence of antibiotic resistance, our world has fallen into a global health crisis. The Tiny Earth Program aims to combat this crisis, by looking for new antibiotics in soil samples found at the UW Green Bay Campus (44.52759°N, 87.92384°W). The soil sample was serially diluted in sterile water and plated onto Luria-Bertani agar (LBA), containing cycloheximide, which selects for prokaryotes. Using the "pick and patch" technique, individual colonies of bacteria were transferred onto LBA and Potato Dextrose agar (PDA) to create library plates. Isolates were screened for antibiotic production against *Bacillus subtilis* and *Escherichia coli* on LBA, PDA, 100% Tryptic Soy agar (TSA), and 10% TSA plates. There was antibiotic production by five isolates. Isolates 5 and 8 exhibited antibiotic production against B. subtilis, displaying narrow-spectrum antibiotic activity while isolates 4,7 and 9 displayed broad-spectrum antibiotic activity against B. subtilis and *E. coli*. Subsequently, gram-staining of the isolates was conducted to determine their gram reaction and morphology. Lastly, colony polymerase chain reaction (PCR) was performed to amplify the 16S rRNA gene, allowing us to use the Basic Local Alignment Search Tool (BLAST) analysis. Through BLAST analysis, the identity of the antibiotic-producing isolates was determined and was consistent with several biochemical tests. This research may be critical to shaping the future of healthcare and to overcome this ongoing global health crisis.

44 Tiny Earth

MARY SIVULA
Northeast Wisconsin Technical College

During this semester my instructor asked the class to join the research Tiny Earth and help find an antibiotic within our local soil. The Tiny Earth project is where students from around the world take soil samples and search for antibiotics from bacteria. Not only does this help our search for more antibiotics, but it also gets us engaged in and learn more about microbiology. I came up with the hypothesis that the soil from my mother's garden would have lots of microbes from decaying plants of the previous years. The first step in our research was to create a soil dilution by pipetting small amounts of our soil into PBS and spreading it along a LB media. After watching the tremendous growth of the microbes, we took samples of the production and placed it onto a new media that would be positioned into a dark box with the same temperature. I concluded that it grew better without light and the number of unique colonies went up. The microbes that grew were then inoculated against the bacteria *Staphylococcus epidermidis* and *Erwinia carotovora* to examine if antibiotic activity was present. The plate containing the microbes showed two zones of inhibition. I then screened my isolate against nine pathogens which showed seven antibiotic producing microbes. This supported my hypothesis on how much the microbes would grow during this project. As we continue to study our research, we are also extending the knowledge of our health care system by joining them in creating new antibiotics to help cure patients in need.

45 Testing the Best Bakery in the World

BRADEN FRIBERG
Northeast Wisconsin Technical College

Antibiotic resistance (A.R.) was discovered almost immediately after antibiotics. To this day many antibiotics are no longer effective because of this. The Tiny Earth project aims to combat A.R. by finding new antibiotics produced by bacteria, as many bacteria are known to fight each other with antibiotics. The experiment is to see if antibiotic producing bacteria can be cultured and identified with a simple method to hopefully use their antibiotics for humans. The experiment began with obtaining a soil sample from Uncle Mike's Bakery in De Pere due to its staple in the community. This dirt was transported to NWTC for a plate culture. Twenty colonies were placed onto a plate for testing against *S. epidermidis* and *Erwinia*. Of those, I identified colony #3 for further testing against cousins of ESKAPE pathogens. These tests showed limited but some antibacterial activity. At the time of writing further testing is ongoing to identify the bacteria's genome, where it could undergo further testing for antimicrobial properties should the genotyping of the 16S RRNA gene show promise. Should this bacterium be identified as new, it could be an exciting frontier of medical science.

46 Hidden Healers: Uncovering Antibiotic Producers Within the Soil

ALLISON DULANSKI
Green Bay West High School

The goal of Tiny Earth is to help solve the global health crisis for antibiotic resistance. Our research helps to solve that problem by studying antibiotic activity in soil bacteria to discover novel antibiotic producers. We started this process by first collecting one gram of soil from the University of Wisconsin Green Bay Cofrin Arboretum. (44.526201, -87.926514). This soil sample was serially diluted using sterile water and plated onto Luria-Bertani Agar (LBA) plates. We then took the unique isolated bacteria colonies and picked and patched them onto one Luria-Bertani Agar (LBA) and one Potato Dextrose Agar (PDA) plate to produce our library plates. These library plates were then utilized in order to spread patch our colonies on tester strands, *E. coli* and B. subtilis, which are gram negative and gram-positive bacteria, respectively. Out of 11 isolates, two were antibiotic producers. Both of these isolates appeared to show antibiotic activity against B. subtilis on all cultivation agar (Tryptic Soy Agar (TSA), 10% TSA, PDA, and LBA). After gram staining it was determined that isolate #5 possessed gram-positive rods and BLAST analysis of the DNA sequence of the 16S rRNA gene and biochemical tests revealed that the genus was *Streptomyces*. This research is valuable because it has the potential to locate previously undiscovered bacteria and novel antibiotics, allowing us to combat the global health crisis involving antibiotic resistance.

47 Broth Versus Agar in Extraction Efficiency, and the Effects of Filtration

JOSHUA CRUZ, EMMA WHITE
Illinois Valley Community College

Due to growing antibiotic resistance, researchers must look for new sources of antibiotics as well as more efficient methods of cultivating them. The main focus of our experiment was to improve extract efficiency as previous attempts yielded poor results. An important secondary purpose was to overcome previous complications with endospore contamination. Three different strains were grown on both broth and agar and separated into trials to compare results. Our original hypothesis predicted that broth would produce better results as the bacteria would have better nutrient availability than the agar; however, this was not the case. We found that there was more antibiotic production when strains were grown on agar. We believe that greater access to oxygen in the plates was more important than any additional nutrient availability from the broth. We also found the use of filters to be extremely effective at removing endospores that survive the extraction process. Trials without filtering were regularly contaminated with endospores that ruined results. In conclusion, our research shows that agar plates along with proper filtration are vital for high yields and preventing contamination.

48 Discovery of Antibiotic-Producing Bacteria in UW-Green Bay Soil

PAIGE PIROCANAC, AASHVI PANDEY
University of Wisconsin - Green Bayzz

Antibiotic resistance is a global crisis, making bacterial infections harder to treat, prolonging hospital stays, increasing healthcare costs, and raising mortality rates. The Tiny Earth program recruits student researchers to combat this challenge by discovering new antibiotics from environmental microorganisms, broadening the search for effective treatments against resistant pathogens. For this project, we collected a soil sample from the UW-Green Bay campus (44.5337059 N, -87.9092881 W), which underwent serial dilution with sterile water. Then it was plated on Luria Broth Agar (LBA) plates that contained cycloheximide to prevent fungal bacteria growth. These plates were incubated at 28°C for 48 hours. Post-incubation, soil bacteria isolates were patched onto Potato Dextrose Agar (PDA) and LBA plates, these were used as master plates for the rest of the experiment. Using the master plates, separate isolates were patched on PDA, 10% Trypticase Soy Agar (TSA), 100% TSA, and LBA plates containing either *Bacillus subtilis* (B. subtilis) or *Escherichia coli (E. coli)*. Four of our isolates showed antibiotic activity in total; 1 against B. subtilis on the 100% TSA plate, 1 against B. subtilis on the 10% TSA plate, 4 against B. subtilis on the PDA plate, and 1 against B. subtilis on the LBA plate. Using Basic Local Alignment Search Tool (BLAST) analysis targeting 16SrRNA, isolates genetic material was characterized, one isolate was identified as *Pseudomonas* chlororaphis. Further identification was performed using the Gram stain technique and various biochemical tests to solidify findings in the experiment.

49 Antibiotic-Producing Soil Bacteria: A Possible Solution to the World's Antibiotic Resistance Issues

TARA DEJARDIN, KARA KAUTZER
University of Wisconsin - Green Bay

A recent study by the Global Research on Antimicrobial Resistance Project on antimicrobial resistance trends over time reveals that more than one million people died each year as a result of antibiotic-resistant illnesses between 1990 and 2021. Our research begins with compost, our initial soil sample was gathered from an area of compost on the UW-Green Bay campus (44.53463 .N, 87.91712 .W). The soil sample was serial diluted in sterile water and plated on Luria-Bertani agar (LBA) containing cycloheximide. Using the "pick and patch" method, isolates were patched onto 10% Tryptic Soy agar (TSA), 100% TSA, LBA, and Potato Dextrose agar (PDA) against both *Bacillus subtilis* and *Escherichia coli*. B. subtilis 100% TSA agar plate yielded antibiotic production for five out of our six isolates which was then used as the master plate. A gram stain was then performed on each isolate, and all isolates were observed to be gram positive. Through basic, local, alignment, search tool (BLAST) analysis and biochemical characterization, it was concluded that isolates 1, 2, 5, and 6 are *Bacillus* genus, and isolate 3 is *Lysinibacillus*.

50 Identifying Antibiotic Producing Non-Soil Bacteria in a 400-Year-Old Log Found in the Omaeqnomenew Maeqtekuahkihkiw

JOSH BESAW
College of Menominee Nation

The Menominee Reservation is known for its vast, dense forest. Bacterial samples were collected from a log on the Menominee Reservation Sawmill which was carbon dated back to over 400 years ago. This study sought to identify if a 400-year-old log could still support antibiotic producing bacteria. The sample was grown on Potato Dextrose Agar using Tiny Earth protocols. Samples were tested using Tiny Earth protocols. The sample was tested against safe ESKAPE pathogens: *Staphylococcus epidermidis*, *Escherichia coli*, and *Bacillus subtilis*. All samples were negative to our safe ESKAPE pathogens, further testing on other safe ESKAPE pathogens is needed to confirm if a 400-year-old log can support antibiotic producing bacteria.

51 Pick and Patching for Humanity

MATTHEW FREITAG, KATRINA HAMMERBERG
University of Wisconsin - Green Bay

The prevalence of bacterial resistance to antibiotics is a growing public health crisis. As bacteria continue to evolve and adapt to existing antibiotics, there is an urgent need to explore innovative approaches to combat them. Our research focuses on discovering new antibiotics produced by soil bacteria. One gram of soil was collected on the UW-Green Bay Cofrin Arboretum trail (44.53900ºN, 87.91900 ºW), serially diluted in water and plated on Luria-Bertani Agar (LBA) with cycloheximide. Once isolated, the strains were screened for antibiotic activity on 10% Trypticase soy agar (TSA), 100% TSA, LBA, and Potato Dextrose Agar (PDA) containing *Bacillus subtilis* or *Escherichia coli* to identify antimicrobial-producing strains. Gram stains, biochemical tests, and the Basic Local Alignment Search Tool (BLAST) analysis of the DNA sequence for the 16SrRNA gene for each isolate were used to identify the genus of each bacterium. Out of twenty isolates, 2, 14, 15, 16, and 17 were antimicrobial-producing strains. Isolates 2 and 17 were effective against B. subtilis and isolate 15 was effective against *E. coli*. Isolates 14 and 16 were broad-spectrum producers against both. By BLAST analysis, it was concluded that isolates 2 and 14 belong to the genus *Pseudomonas* and isolate 16 belongs to the genus *Microbacterium*. This was supported sufficiently by the gram stain results. By identifying and characterizing strains known for their antimicrobial properties, we aim to reveal new antimicrobial compounds that can effectively treat infections in new ways.

52 Antibiotic Production in High Moisture Soil

KATHRYN KAUFMAN, NICOLE O'DELL (WAAGE), AMBER PLOSCZYNSKI
Northeast Wisconsin Technical College

Antibiotic resistance is becoming a crisis across the world due to misuse and overuse of antibiotics. If new discovery does not occur within the next few decades, mortality rates could significantly increase. The Tiny Earth Project presented an opportunity to research soil in different communities and environments all over the world in hopes of finding new antibiotic releasing microbes. Our group chose to collect soil from a variety of different environments all of which have a high moisture content. We collected samples from Sherwood Forest Park, Bairds Creek, and a family-owned chicken coop. Our hypothesis was that with the high moisture content of our soil would contain many microbes releasing antibiotic properties. To start our experiment, we diluted the samples and plated them using the streak plate method. The microbes discovered were inoculated and tested against bacteria *S. epidermidis* and *Erwinia* to examine if any microbes would be able to produce antibiotics. Baird's Creek and Sherwood Forest Park had one possible antibiotic-producing microbe while the chicken coop had two possible antibiotic producing microbes. We then chose one antibiotic producer and tested them against relatives of the ESKAPE pathogens. All three of our results showed one antibiotic producing microbe. A PCR test was then completed and showed all three were also positive for the 16s rRNA gene and were sent for DNA sequence testing. Our hypothesis was correct as we all did find antibiotic-producing microbes in our samples. Our research of Sherwood Forest Park, Baird's Creek, and the family-owned chicken coop's soil is ongoing, some results are yet to be determined.

53 Discovering Antibiotic Producers in Urban and Agricultural Soils

MELANIE HERB, DANIELLE MUELLER
Northeast Wisconsin Technical College

With this problem in antibiotic-resistant bacteria, finding new sources of antibiotics is so important. As part of the Tiny Earth project, we collected soil from two separate locations, a farm environment and urban environment, to see if either would have microbes that produce potential antibiotics. We believed that farm soil would contain more antibiotic-producing microbes because it is more nutrient-rich and the soil comes from a field that rotates crops each season. This year was sweet corn, last year was different. The tilling and cow manure improves the soil's quality. On the other hand, the urban soil might have less due to little nutrients and pollution. In the lab, we diluted and plated our two soil samples taken from the two different environments using the Streak Plate Method, then we screened the isolated microbes against the bacteria's *Staphylococcus epidermidis* and *Erwinia*, to see if any samples had antibiotic properties. At first, we found one possible antibiotic-producing microbe from each soil sample, through further testing, we examined our antibiotic producers against the ESKAPE pathogens, but no antibiotic growth was shown in either sample. Our hypothesis was not supported, as both environments contained a similar number of antibiotic-producing microbes, with little to none detected. Our research on antibiotic-producing microbes in both our farm and urban environment is still ongoing, so further results are yet to be determined.

54 Bacterial Associations My Mycelial Affiliated Soils

BENNETT SHIRING
Madison College

Mycelial networks play a large role in most soils, often associating with the root networks of plants for mutual benefit. We collected soil samples from the base of fruiting bodies of mushrooms, as well as one sample collected from the base of a ghost flower, a mycotrophic plant that parasitizes mycorrhizal networks for all its energy. We also collected control samples from spots nearby to each fruiting body. We then made spread plates to collect bacterial colonies from each condition, which were then screened for antagonism against known bacterial and fungal pathogens. We compared proportions of zones of inhibition across experimental and control conditions and found greater antibiotic production in selected mycelial associated soils. This might suggest that soil bacteria associated with mycelial networks (or where mycelial networks are in higher concentration) might have more utility in the hunt for antibiotics.

55 Traveling to Parks Around Wisconsin

GWEN STRYZEWSKI, AZLLYN VAN DRISSE, ALICIA VARGAS
Northeast Wisconsin Technical College

For the last century antibiotics have been discovered and able to cure bacterial infections, but now we have been faced with a crisis with lack of new findings of antibiotics. The Tiny Earth Project has given us the opportunity to collect and research soil from our different communities to try to end this drought and discover a new antibiotic. While running this experiment, we hoped to find a new antibiotic to help end the crisis. We have used many methods to complete our research and contribute to possible new findings. Our first step was collection. Our group decided to collect soil samples from public parks throughout the state of Wisconsin. The parks we decided to collect soil from were Josten Park, Bellevue, WI, Silver Creek Park in Manitowoc, WI, and Coleman Community Ball Park, WI. Two of our group members collected surface soil while one collected rhizosphere soil. After sample collections, we began by diluting our samples to prepare for culturing. The culturing process involved isolating our samples to allow bacterial colonies to form on agar plates. We continued our process by changing the conditions of our cultures in hopes of acquiring change or growth in our samples. After this process, we created master plates with 20 colonies to test against gram positive and negative bacteria. These colonies were then isolated and those that demonstrated activity were selected for testing with relatives of ESKAPE pathogens. The colony that demonstrated resistance against the relatives of ESKAPE pathogens was then isolated for PCR. All of our organisms had similar outcomes on the tests we ran. Our organisms all produced antibiotic agents that were active against gram-positive bacteria and cocci shaped bacteria. However, one organism did have some activity against gram-negative bacteria. Even though we all collected soil from different parks throughout the state of Wisconsin, we were able to find similar antibiotic-producing bacteria in our soil samples. As we continue this project, we hope to discover a new antibiotic that could change the future.

56 Antibiotic Report on Flowerbed Soil

BRANDON STEPHENS
Northeast Wisconsin Technical College

In recent years, a conundrum has presented itself in which many pharmaceutical companies are not providing ample funding and effort into the research required to synthesize new antibiotics to combat the bacteria that have evolved to resist the current antibiotics. As such, an organization known as Tiny Earth is seeking the aid of colleges, such as NWTC, to assist in obtaining and researching antibiotic producing bacteria that could be integral to further development of antibiotics. Our class has been tasked with analyzing samples of soil from various areas to locate these bacteria. I hypothesized that a moderate amount of antibiotic activity, and, therefore, antibiotic producing microbes would be present in the sample of soil I collected. We individually gathered soil and recorded information about the area of extraction. The bacteria from these soil samples were cultivated on solid culture media petri dishes and tested against safe ESKAPE pathogens to view any development of macroscopically visible antibiotic activity. Additionally, PCR was used to acquire more strands of DNA for gel electrophoresis in an attempt to uncover the identity of the species of bacteria cultivated. The results displayed that no antibiotic activity from the bacteria in my sample of soil was observable against the eight tested-for safe ESKAPE pathogens. In conclusion, my hypothesis has been rejected since no antibiotic producing bacteria were collected from the sample of soil I had gathered, and further testing on soil from homes in city neighborhoods is required.

57 The Waters of Wisconsin

JULIA WILLIAMS, RIVER FLYNN, TIFFANY POQUETTE, JENNA VAUGHAN
Northeast Wisconsin Technical College

Over 100 hundred years ago one of the greatest discoveries in medical history was discovered, antibiotics. But, over time bacteria have become antibiotic resistant. This has caused a halt in modern day medicine, and we are running out of solutions. The Tiny Earth project has brought this issue to colleges all over the country to help find a solution to our antibiotic crisis. To determine if each of our soil collections contained any antibiotic producing components, we collected our soil samples from places near a body of water. As a group, we believed that soil located near bodies of water would produce more antibiotic producing microbes. After our soil collection, we needed to dilute our soil samples so that we were able to identify single colonies of bacteria. Secondly, we had to choose two new conditions which would determine if our samples grew more bacteria in a specific setting. Results varied for our samples based on which two conditions we chose. Furthermore, we decided to test how our bacteria samples grew against *Erwinia* and *S. epidermidis*. This method involved utilizing 20 colonies from our original plates and placing them on the plates that were spread with *Erwinia* and *S. epidermidis*. Additionally, we completed a streak plate method which allowed us to conclude which of our bacteria showed activity against relatives of ESKAPE pathogens. Lastly, we completed a PCR lab. This lab was used to find the specific DNA sequence of each of our bacteria. Our research with our samples is still in the process of determining whether our samples contain antibiotic producing microbes!

58 Antibiotic-Producing Bacteria Near Water Sources

TABITHA ZUELKE, KAYLA BAYS, SKYE DERKS, ANGELA LOZA
Northeast Wisconsin Technical College

Antibiotic resistance in bacteria is a growing global health issue, contributing to increased mortality and limited treatment options for infections. Resistance is developing faster than new antibiotics are discovered, pushing researchers to explore alternative sources of antibiotic-producing microbes. Through the Tiny Earth project, we aimed to locate and isolate different soil bacteria capable of producing antibiotic properties, hypothesizing that soil near various water sources might host diverse bacteria with antibiotic potential due to possible high microbial activity and diverse environmental conditions. We predicted that the natural spring would show most antibiotic activity, as the presence of small worms seen in the sample suggested a high microbial environment. To test this, soil samples were gathered from near a creek, river, natural spring, and waterfall. We diluted and plated the samples to isolate bacterial colonies and cultured them under different chosen conditions. We each chose 20 different colonies from our samples and screened them against S.epidermidis and *Erwinia* to identify any antibiotic activity. While the waterfall showed zero activity, the natural spring, river and Creek each showed antimicrobial activity. Further testing was done with one active colony we chose to test against the ESKAPE pathogens which revealed no additional activity for any of the samples. These findings suggest that soil bacteria near water sources may produce some antibiotics, although effectiveness varies by pathogen. We concluded that our hypothesis was proven to not be accurate. While further results from our samples are pending, the testing is still ongoing and being conducted.

59 Antibiotic Producing Bacteria from Suamico River Soil

MARIANA TKACHUK
Northeast Wisconsin Technical College

Living in a post-antibiotic era, society came to the problem of antibiotic resistance. It is a problem because many antibiotics do not work anymore. If not resolved, this problem could lead to previously treatable infections becoming deadly. The Tiny Earth Project relates to this issue because by doing it we were looking to find antibiotic-producing microbes. I predicted that my soil sample would contain two antibiotic-producing microbes. To address the problem, a sample of soil from the Suamico River was collected. I chose to get the soil from the Suamico River because there are a lot of animals that are walking around and there is a smell that can indicate that a lot of bacteria live there. After the soil was collected, it was diluted and spread on plates for bacteria to grow. After bacteria grew on plates, they were put in new conditions to see if other bacteria would grow there. Twenty bacteria were chosen to be tested for antibiotic activity on tester plates against *S. epidermidis* and *Erwinia*. The testing showed that five bacteria had activity against *S. epidermidis*, however, no bacteria was active against *Erwinia*. One bacteria was chosen to be tested against the other eight bacteria on the streak plate. The bacteria showed activity against *Bacillus*. There was a trend noticed since both *S. epidermidis* and *Bacillus* are gram-positive bacteria, so the chosen bacteria tend to have activity on gram-positive bacteria. Antibiotic-producing bacteria were found during this project. For now, it is unknown if the bacteria has already been studied or if it is a new bacteria, so further tests have to be done.

60 Are Our Kids Safe on the Green Isle Park Soil?

ANGELA BEBEAU
Northeast Wisconsin Technical College

Our society has been abusing the use of antibiotics, creating multiple resistant bacteria. The goal of this research is to raise awareness about antibiotics resistance and educate the population on the importance of the correct use of antibiotics. My hypothesis was that I would find a new antibiotic producing microbe. My research at Green Isle Park in Green Bay. I chose this location because this specific location goes underwater during the heavy raining season creating great resources for microbes to grow and is near this location a playground is located where children play every day. I obtained a sample of the subsoil, diluted it, and plated it using the streak method. Once colonies formed, I changed the conditions (temperature and nutrients) to see if new microbes would grow, I discovered the microbe preferred the water. From here I inoculated the microbes and exposed them to 2 different bacteria: *S. epidermidis* and *Erwinia*. The results showed I had 2 microbes that were double producers. I exposed one of them to the ESKAPE pathogens. This last test showed me that my bacteria worked against two gram negative types, *bacillus* bacteria with no grouping. My hypothesis so far is true as I found an antibiotic producing microbe, however my research is still ongoing to find out exactly if this is a new microbe or not.

61 Backyard Curiosity

CASSANDRA ANDRESEN
Northeast Wisconsin Technical College

Ever wonder what is sneaking around in your backyard? There is more than just wildlife and trees; there are microbes too. Some microbes can produce antibiotics, but recent quantities have been lacking. Antibiotic resistance has become a global crisis in recent years. The disease-causing pathogens are evolving faster than we can find medicines for them. To be a part of the solution to a continuing crisis, I've decided to take the opportunity provided by The Tiny Earth Organization to research a soil sample. My research is conducted on a sample from my back yard out of pure curiosity. I diluted the soil and put it on LB plates using the streak method. I then chose 20 sample sites and tested the bacteria against *S. epidermidis* and *Erwinia*. Out of 20 samples, only one bacteria had a reaction to *S. epidermidis*. The one sample that showed activity was then tested on the ESKAPE pathogens. Surprisingly, my bacteria sample was active against 5 out of 8 ESKAPE samples. My hypothesis was inaccurate, as I thought there would be no activity since the environment is overgrown and dry. Further testing is going to be conducted; therefore, results are still pending.

62 Fishing For Antibiotics

MYA HOLM
Northeast Wisconsin Technical College

Globally we have come face-to-face with a serious problem called antibody resistance, which means that common illnesses like strep throat could be fatal once again. Our job as NWTC students is to work with the Tiny Earth organization and gather samples of soil from various locations to potentially discover a new bacteria that could lead to the development of a new antibiotic. For my soil sample I decided to choose the Green Bay Yacht Club as it was a location that was never previously tested on, and it was by a body of water. My hypothesis stated that the Green Bay Yacht Club soil sample would contain at least 3 different microbes containing antibiotic properties. The method we used to dilute our soil sample and separate the bacteria was called the streaking method. Once we isolated the bacteria from our soil sample, we then tested them on a master and tester plate containing S.epidermidis or *Erwinia* to see which bacteria sample produced antibiotics against those bacteria. Once this was completed the Green Bay Yacht Club showed 2 different producers for antibiotic microbes. After comparing the two antibiotic producing bacteria, I then had to choose one to screen against the ESKAPE pathogens which revealed additional activity against *B.subtilis*. This concluded that my hypothesis was incorrect, however. Our research is still ongoing; therefore, some results are to be determined.

63 Antibiotic Production in Wetland Soil Bacteria

EMMA GEIB, ETHAN WITCHPALEK
University of Wisconsin - Green Bay

Antibiotic resistance is becoming more and more common while antibiotic discovery lags behind. Our research aims to find new antibiotics by studying bacteria in soil. Our research began by collecting soil from a swampy wetlands type forest 30 minutes south of Green Bay (44º15'11" N, 88º1'41" W), as wetland soil has a high diversity of bacteria. One gram of soil was serially diluted and plated on LB agar containing cycloheximide. Isolated colonies were plated against gram-positive *Bacillus subtilis* (B. subtilis) and gram-negative *Escherichia coli (E. coli)* to screen for antibiotic production. Three narrow spectrum antibiotic-producing colonies with activity against B. subtilis were discovered. Through sequencing the DNA for the 16s rRNA gene, biochemical tests, our antibiotic producing bacteria belong to the *Bacillus* and *Lysinibacillus* genera. The outcome of our research could result in new antibiotics in the future.

64 Mysterious Soil Microbes

ALYSHA KLUG
Northeast Wisconsin Technical College

The microbes are pushing back! We are facing a real concern as antibiotic resistance becomes more prevalent. Due to overuse and misuse of antibiotics, bacteria are evolving and becoming increasingly resistant to antibiotics. Pharmaceutical companies have not released new antibiotics for several decades. Tiny Earth allows students to be part of the antibiotic discovery initiative by testing soil in hopes to find antibiotic producing bacteria. I collected my soil sample from my hobby farm in Morrison, Wisconsin in an area where chickens and cows have grazed and where many plants are currently growing. I hypothesized I would find one antibiotic producing bacteria in my soil because of the variety of plants competing for nutrition in the area. After soil dilution and initial plating onto LB Agar, I selected new culture conditions and plated my dilution onto PDA and TSA. The PDA promoted the growth of mold, and my sample had what appeared to be Penicillin droplets which was an exciting observation.

Additional tests were performed, and my selected bacteria did not show signs of producing antibiotics. Further testing is being completed to identify the bacteria.

65 Pigs, Parks, and Parking Lots

KATELYN FISHNICK, MARISSA KELTESCH, KAITLYN MIOTKE
Northeast Wisconsin Technical College

Approximately 1.2 million people die annually from infections that have become resistant to all antibiotics. With antibiotic resistance on the rise, it is more urgent than ever to tackle this challenge. Our team is seeking new antibiotics to effectively treat resistant infections. We each harvested a soil sample from distinct environments; a farm, a park, and a parking lot. We hypothesize that we would discover six new bacteria with antibiotic properties. We diluted the soil to minimize background biota, and isolate colonies of bacteria using the streak plate method. We tested 60 different bacterial isolates against bacterial *S. epidermidis* and *Erwinia*. Then, each of us inoculated one bacteria within the ESKAPE pathogens and found that we had four bacteria with antibiotic potential. Our initial hypothesis was incorrect. Our research is still ongoing at this time, leaving some results to be determined.

66 Exploring Antibiotic Producers in the Soil of a State Park

COURTNEY SALVESON-KREPLINE, SALEM OHIZU
University of Wisconsin-Green Bay

Antibiotics have long been used to treat major infections, leading to the development and discovery of many different antibiotics over the years. However, alongside these advances, antibiotic-resistant bacteria have emerged, posing a significant threat to global health. With traditional pharmaceutical efforts in decline, it has become crucial to explore new avenues for discovering antibiotics. Our goal in this research project is to investigate a unique environment in the hopes of identifying novel antibiotics that could address this growing resistance crisis. We chose to investigate the soil at High Cliff State Park at the coordinates 44.163250, -88.289008. To begin with, we isolated the bacteria by performing tenfold serial dilutions and plating the dilutions onto LB agar plates containing cycloheximide. To assess their antibiotic activity, soil isolates were patched to agar plates containing *Bacillus subtilis* or *Escherichia coli*. Of the six isolates, four displayed antibiotic activity and underwent further analysis with gram staining, biochemical tests and BLAST analysis of the DNA sequence for the 16S rRNA gene. Two antibiotic-producing isolates belong to the genus *Streptomyces*, and two belong to the genus *Pseudomonas*. These findings support the importance of exploring unique soil environments as a source for new antibiotics. Such discoveries could enhance treatments for bacterial infections and aid in the fight against superbugs like *Clostridioides difficile*. This project demonstrates the role of student led academic research initiatives, like Tiny Earth, in addressing the global antibiotic crisis.

67 Finding Novel Ways to Solve the Antibiotic Resistance Crisis Through Research of Antibiotic Production in Soil Bacteria

ELLA PATTY
University of Wisconsin-Green Bay

The golden age of medicine gave way to an era of antibiotic-resistant bacteria. The Tiny Earth program seeks to address this public health emergency by screening for novel antibiotic activity in soil bacteria. A soil sample was collected along the arboretum trails of the University of Wisconsin-Green Bay at 44.52734°N, -87.92532°W. One gram of soil was serially diluted in sterile water and plated onto Luria-Broth (LBA) agar with cycloheximide to select for soil bacteria. Master plates were created by 'picking and patching' isolated soil bacteria on LBA and Potato Dextrose Agar (PDA). The samples were screened for antibiotic activity by patching soil isolates on plates of 10% Tryptic Soy Agar (TSA), 100% TSA, PDA, and LBA containing either *Escherichia coli* or *Bacillus subtilis*. Gram stains, biochemical assays, and the Basic Local Alignment Test (BLAST) analysis of the DNA sequence for the 16SrNA gene were conducted to identify the genus of each antibiotic producer. Isolates 3, 4, and 7 showed narrow antibiotic activity against B. subtilis on PDA, 10% TSA, and 100% TSA, and 3 and 7 were characterized as gram-positive rods and isolate 4 as a gram-positive coccus. The antibiotic-producing bacteria identified will be studied and used in the development of novel medications.

68 From Clay Soil to Antibiotic Resistance: Overcoming Medical Challenges

PAIGE HANSON AND MAYCEE DETTMAN
University of Wisconsin - Green Bay

Antibiotic resistance is an increasingly dangerous public health crisis in today's world and eventually will be the number one cause of death. The aim of Tiny Earth research is to screen soil bacteria for novel antibiotic activity. A soil sample from Legacy Nature Reserve – Clay Banks was collected at [44.72918 N, 87.34273 W] in an area clay deposit. One gram of bacteria was serially diluted in water and plated on Luria-Bertani agar (LBA) containing cycloheximide to isolate bacteria. Six isolates were tested for antibiotic activity against *Escherichia coli* and *Bacillus* subtilis (B. subtilis). Four isolates showed antibiotic activity against B. subtilis. This activity was apparent on 100% Trypticase soy agar (TSA), 10% TSA, and LBA plates. All four isolates are gram-positive, spore producing rods. According to Basic Local Alignment Search Tool (BLAST) analysis of the DNA sequence for the 16SrNA gene, isolates #1, #2, and #5 belong to the genus *Bacillus*.

69 Antibiotic Producers Collected from Goat Farm

OLIVIA PRICE
Northeast Wisconsin Technical College

The issue of antibiotic-resistant microbes has been an ongoing problem for scientists. Due to limited antibiotics and the increase of antibiotic resistance, we have seen an increase in mortality due to serious infections. The Tiny Earth organization gives students the chance to research known antibiotics, as well as the opportunity to discover new antibiotics. My soil sample was collected from a small goat farm which raises meat goats for consumption. I chose this location in hopes that there are microbes that release specific antibacterial agents in the environment the goats inhabit. My hypothesis is that I will discover a new antibacterial producing microbe as well as further my knowledge of known microbes within the soil in the goat's main environment. For this experiment, I obtained a sterile soil collection from the goat farm which I then diluted and spread the mixture onto separate labeled plates. From there I took 20 isolated colonies from the array of soil dilution plates and placed them onto 3 new plates with new conditions. The three new plates contained Erwina, *S. epidermidis*, and a master plate with neither gram type as my control. Three of my isolated colonies had activity against both bacteria making them double-producers. Out of the three double-producers I chose one colony and made an all-tester plate with the ESKAPE pathogens and a streak plate of my chosen colony. The ESKAPE all-tester plate had antibiotic activity against *Acinetobacter* and E.coli, both of which were gram-negative bacteria. I then proceeded to take a sample from the streak plate and combine it with PCR master-mix in a PCR tube for further testing.

70 Antibiotic Producing Soil Collected from the Suamico River

MADISON GOFFINET
Northeast Wisconsin Technical College

Living in a post-antibiotic era, society came to the problem of antibiotic resistance. It is a problem because many antibiotics do not work anymore. If not resolved, this problem could lead to previously treatable infections becoming deadly. The Tiny Earth Project relates to this issue because by doing it we were looking to find antibiotic-producing microbes. I predicted that my soil sample would contain two antibiotic-producing microbes. To address the problem, a sample of soil from the Suamico River was collected. I chose to get the soil from the Suamico River because there are a lot of animals that are walking around and there is a smell that can indicate that a lot of bacteria live there. After

S. epidermidis, however, no bacteria was active against Erwinia. One bacteria was chosen to be tested against the other eight bacteria on the streak plate. The bacteria showed activity against Bacillus. There was a trend noticed since both S. epidermidis and Bacillus are gram-positive bacteria, so the chosen bacteria tend to have activity on gram-positive bacteria. Antibiotic-producing bacteria were found during this project. For now, it is unknown if the bacteria has already been studied or if it is ant bacteria, so further tests have to be done.

71 Sneak Peak in the Life of Being a Scientist

MEE XIONG
Northeast Wisconsin Technical College

With the mission of Tiny Earth, we can experience what it is like to be a scientist conducting research about antibiotic crisis, soil crisis, and antibiotic discovery. The crisis we have today with antibiotics is that bacteria have evolved and become more resistant to the current medications. Meaning it is getting harder to treat infections. This resistance is currently affecting the world by causing more deaths and complications. For our soil, mother nature's natural resource, is being depleted from its nutrients. Making us lose bacteria that could be used to save our lives. Since our soil can contain microbes that produce antibiotics. Due to pharmaceutical companies believing that the investment is too low. The research to find new antibiotics has been delayed. Thanks to Tiny Earth, we get to conduct our own research with our soil samples to find out whether we find antibiotic producing microbes. I chose to take a soil sample from my front yard since I have a big, beautiful maple tree and my lawn is always green in that area during the summer. I then diluted and plated my soil using the streak plate technique. The microbes found were inoculated and screened against the bacteria S. epidermidis, Erwinia, and safe-ESKAPE pathogens to see if my microbe produced any antibiotics against the bacteria and it did not.

72 Antibiotic Producers Collected from Granddad Bluff

MADISON BIESE
Northeast Wisconsin Technical College

For decades, scientists have struggled with antibiotic resistant pathogens and the challenge of discovering new antibiotics. This results in an increase of mortality rates in our country. The Tiny Earth organization presented the opportunity to research soil to find new microbes that release antibiotics and present my findings to the conference. I went to La Crosse Wisconsin and searched for a natural environment where animals run and the rain falls. My hypothesis is that Granddad Bluff will have five microbes that produce antibiotics. This is because Granddad Bluff goes through all four seasons and is a clean environment. Nature grows and animals run through these bluffs so I feel that the natural environment will produce a great amount of antibiotics. During class, I diluted and plated the soil from my sample taken in La Crosse using the streak plate method. The microbes I found were then screened against S. epidermidis and Erwina to see which microbe produces antibiotics against the bacteria presented. I found a total of three microbes that produced antibiotics. I then chose one of my producers and screened it against the ESKAPE pathogens. My results showed no further antibiotic producing microbes. My hypothesis was not accurate, as my soil sample only produced three antibiotic producing microbes instead of five. My research on Granddad bluff antibiotic producing soil is ongoing, and therefore some results are to be determined.

73 Possible Antibiotic Producer Collected from Biodiverse Woodland in Town of Two Rivers, WI

MICHELLE EIS
Northeast Wisconsin Technical College

Due to antimicrobial resistance from the overuse and misuse of antibiotics, new antibiotic-producing microbes must be discovered. Tiny Earth, the founder of a sustainable, grassroots network of institutions, engages students in the search for novel antibiotic-producing microbes through research, increasing the opportunity to find possible isolates for further development. The site selected for collecting a soil sample was a privately owned, biodiverse woodland, located in the Town of Two Rivers. My hypothesis was that a biodiverse environment would contain a diverse population of bacteria and thus increase the chance of discovering at least one bacterium with antibiotic-producing properties. The soil sample was diluted and plated using the streak plate technique. The microbes found were inoculated and screened against gram-positive Staphylococcus epidermidis and gram-negative Erwinia sp. Isolate #10 from the streak plate was found to react with S. epidermidis. Isolate #10 was then screened against the safe cousins of the ESKAPE pathogens, including S. epidermidis. The results were inconclusive because S. epidermidis appeared to either have not grown or experienced large enough clearing to lyse S. epidermidis completely. A PCR test displayed bands associated with base pairs of the control. Identification of Isolate #10 from the PCR test is pending. Further biological testing will be performed to identify Isolate #10 and to determine whether the results support or deny my hypothesis. Bluff will have five microbes that produce antibiotics. This is because Granddad Bluff goes through all four seasons and is a clean environment. Nature grows and animals run through these bluffs so I feel that the natural environment will produce a great amount of antibiotics. During class, I diluted and plated the soil from my sample taken in La Crosse using the streak plate method. The microbes I found were then screened against S. epidermidis and Erwina to see which microbe produces antibiotics against the bacteria presented. I found a total of three microbes that produced antibiotics. I then chose one of my producers and screened it against the ESKAPE pathogens. My results showed no further antibiotic producing microbes. My hypothesis was not accurate, as my soil sample only produced three antibiotic producing microbes instead of five. My research on Granddad bluff antibiotic producing soil is ongoing, and therefore some results are to be determined.

74 Tiny Earth

TAYLOR KANACK, AMY GUZMAN AND SAM SERVAIS
Northeast Wisconsin Technical College

With new antibiotics being scarce and issues with antibiotic resistant pathogens forming, finding new bacteria that produces antibiotics has become essential. The Tiny Earth Project gives students the opportunity to research soil within their communities to try to find new bacteria that produce antibiotics and to present any of our data with others. Together We have gathered soil samples from different areas in our communities which included the backyard next to an agricultural field, Fritsch Park by the playground, and Fritsch Park hiking trail by a fallen tree with mushrooms. It was hypothesized that the sample from the backyard would produce two antibiotic producing bacteria, the sample from the playground would produce one, and the sample from the hiking trial would produce five. Our methods included soil dilution, culture change of conditions, and testing the soil bacteria against the following organisms S. epidermidis, E. coli, Acinetobacter, Pseudomonas, Enterobacter, Bacillus, Mycobacterium, Lysobacter, and Erwinia. The sample taken from the playground showed activity against Mycobacterium; the hiking trail and backyard samples showed activity against Bacillus. This is still an ongoing experiment that is still undergoing more testing at NWTC.

75 Are You Eating Antibiotic-Resistant Bacteria?

ISABELLA CARLSON, KADEN GAEDTKE, HEIDI SMANEY, ADRIANA GARCIA
Northeast Wisconsin Technical College

Antibiotic-resistant pathogens have become more prevalent over the past few decades because of the misuse of antibiotics by humans. A new and effective antibiotic has not been developed since the 1990s and within this project, our goal is to discover a new antibiotic-producing bacteria. These findings could lead to the development of a new antibiotic which will help slow the continued rise of mortality from antibiotic-resistant infections. To combat this issue, the Tiny Earth organization has allowed students to participate in soil research throughout our communities. Students were given freedom to choose an ideal location in which they believed would contain antibiotic producing bacteria in the soil. Our group chose to extract soil from nutrient-rich environments that food is locally sourced from. We believe that because bacteria compete for resources in nature and can produce antibiotics as a survival mechanism, there may be high amounts in these areas. Heidi and Adriana took samples from personal home gardens, and Isabella and Kaden took them from the Fox River and the Bay of Green Bay. We diluted and plated our four soil samples using the streak plate method and grew isolated colonies of bacteria that we screened against S. epidermidis and Erwinia bacteria. Kaden and Bella each only found one antibiotic producer, and Heidi found one that showed activity but stopped after 24 hours. Adriana found none and continued the experiment using Heidi's chosen microbe. These microbes were then screened against ESKAPE pathogens that unfortunately showed no activity, proving our initial hypothesis wrong. Testing is still ongoing and could still display new results.

76 Antibiotics: Because Germs Aren't Going to Fight Themselves

NOEL COTTO LEBRON,HAILLE SEIDLER
Northeast Wisconsin Technical College

To date, most antibiotics originate from soil microbes that produce compounds capable of eliminating pathogens that are resistant to multiple drugs. The Tiny Earth Project engages students in the search for new antibiotics from soil, which could help fight pathogens that have developed resistance to existing drugs. At the start of the project, our group predicted that each of us would find one antibiotic producing bacteria. Our soil collection sites include two areas of the Fox River and the Green Bay Correctional Institution. Methods we used to address this problem include collecting a soil sample and bringing it into the lab. Following, a soil dilution was performed and plated onto LB media. From there, we then selected different kinds of bacteria to continue with our research. Afterward, to determine if it affected the type of bacteria that grew or altered the way the bacteria grew, these bacteria were subjected to two environmental conditions that correspond to the site of collection, such as tryptic soy agar and a Luria-Bertani broth at 37 degrees Celsius. With that, we selected one bacterial colony to proceed with and tested it against the S. epidermidis, Erwinia, and ESKAPE pathogens considering those findings. Each of us discovered one microbe that produced antibiotics. Research is still ongoing; therefore, some results are still to be determined.

77 Antibiotic Producers Found at Local Park and Ride

JACOB PELKOLA
Northeast Wisconsin Technical College

Due to the rise in antibiotic resistant pathogens, scientists and healthcare professionals around the globe have seen an increase in mortality rates due to these pathogens. The Tiny Earth Organization tasked us with collecting our own soil samples to potentially find new antibiotics. I took my soil sample from a park and ride that has been full of garbage for years. I hypothesized that due to the dirty state of the park and ride it would be a suitable environment for bacteria to thrive. I figured I would find around twelve antibiotic producers. In the lab I used the soil dilution method and then the streak plate method to inoculate the microbes. With the inoculated microbes, I then tested them against Erwinia and S.epidermidis. I had one bacteria that worked against Erwinia. I then tested it against ESKAPE pathogens which yielded no dual producers. My hypothesis was incorrect since the park and ride had yielded one antibiotic producer. My research is still ongoing and further results are pending.

78 Tiny Earth

JASON CAVALLIN
Northeast Wisconsin Technical College

The continuing rise of antibiotic-resistant microbes necessitate the need for new forms of antibiotics to combat these "super bugs". As the number of antibiotic-resistant diseases increase, so too does the need for new forms of medications capable of combating them. By working to discover new forms of antibiotics we can continue to treat patients afflicted by diseases that resist traditional antibiotic treatments currently available. For this experiment I collected a soil sample from a local area with an existing slime mold colony in the hopes that the microbes in the ground would be more likely to exhibit antibiotic properties. I then isolated and cultivated various microbes from the soil sample, eventually narrowing my experiment to a single microbe colony. After exposing my microbe colony to a variety of known disease-causing microbes, I found that my soil sample contained 3 microbes that were effective against S.epidermidis and Erwina, however only one sample was effective against both, and this was the colony that I selected for the final experiment. I found that my antibiotic microbe was also effective against Acinobacter after the ESKAPE experiment. Having found an antibiotic colony within my soil sample that is effective against 3 tested disease-causing microbes, I hope to use this microbe to further the development of new antibiotics to combat the rise of "superbugs".

79 Antibiotic Producers from the Lake Michigan River Systems

JACOB ARCEO, JAMES JOHNSON
Northeast Wisconsin Technical College

Scientists around the world have been trying to solve a problem that has been affecting how we treat illnesses and fight off pathogens. Antibiotic resistance has been a roadblock towards stopping bacteria from growing and reproducing. Because of this, the world has been affected due to higher mortality rates and an increase in infection. The Tiny Earth project has opened new pathways to understand this ongoing problem and has opened doors towards possibilities of creating a new antibiotic. To achieve this, we have collected soil from two points. The first being before the entry into Lake Michigan in the Peshtigo River. The other out of the Fox River which merges from the lake to find bacteria that produce antibiotics. Our hypothesis was that the fox river location would be a stronger producing area for these bacteria due to the higher population and exposure to more poultice through the lake and high city traffic. Our microbes were tested against S. epidermidis and Erwinia. It was found that the Fox Valley location had one antibiotic producer against Erwinia while the Peshtigo River had none. Therefore, our hypothesis was proven to be correct, and we proceeded with the one antibiotic producer we had. Testing it up against the ESKAPE pathogens finding producers against E. coli and Acinetobacter. As of right now our research is ongoing and we have moved on to PCR testing for further review.

80 Antibiotic Producers: Farmland versus Healthcare Facility

CAILEY MASSART, LAUREN GEE
Northeast Wisconsin Technical College

Over many years, our world has been faced with a deadly problem. Antibiotics are no longer working against certain bacteria, which is known as antibiotic resistance. If we don't find a solution fast, something as simple as the common cold could be fatal, increasing mortality rates and possibly leading to human extinction. We had the opportunity to take part in the Tiny Earth project, which is trying to find new antibiotic-producing bacteria from soil samples. We chose our locations because we wanted to compare animal populated soil with a location that has a lot of human traffic. We predicted that farmland would have more antibiotic producers, because there is less human disturbance to the soil. We diluted our soil and placed it on a streak plate, then spread our soil on new media. We then spread Erwinia and S. epidermidis on two new plates, then inoculated our diluted soil sample on the plates to see if they would grow any antibiotic-producers. We chose one singular colony from each location to continue with and screened it against the ESKAPE pathogens. The healthcare facility soil showed activity against one of the pathogens, while the farmland soil showed activity against three pathogens. We are now using genetic testing to see if we can identify our bacteria or if we discovered a new bacterium. Our hypothesis was correct, as the farmland soil grew more antibiotic-producing bacteria. Our research is still, ongoing; therefore, we do not have all of our test results yet.

81 The Tiny Earth Within The Soil Of A Psychiatric Hospital

CLAIRE GEURTS
Northeast Wisconsin Technical College

There are countless diseases caused by bacteria, and for a while, antibiotics worked to treat these diseases. However, we have entered an era of antibiotic resistance. More and more bacteria are resistant to the antibiotics we have, and antibiotic development has slowed dramatically. To address this global challenge, new antibiotics must be discovered, and the Tiny Earth organization does this through student research. So, soil was taken and researched to find new antibiotic producing microbes. Soil was collected from the courtyard of Willow Creek Behavioral Health – a local behavioral health hospital. Since this courtyard is accessed regularly by 70+ patients and is often used as a smoking area for those patients, I predicted an antibiotic producing microbe would be present. The soil was diluted and plated, and the microbes that grew were then inoculated and screened against the bacteria S. epidermidis and Erwinia. After the bacteria were screened against the two bacteria, I discovered the sample did not contain any antibiotic producing microbes. Further testing is ongoing to determine the identity of the bacteria I did find.

82 Semi Aquatic Antibiotic Producer

RAMIRO CORTEZ, DYLAN CLOUD
University of Wisconsin - Green Bay

Antibiotic resistance is currently a pressing public health crisis, which has contributed to the loss in life of many individuals and has cost the healthcare system billions of dollars. The Tiny Earth Initiative aims to address this emerging threat by conducting research to find novel antibiotics. A soil sample was collected from Baird Creek (44.50546° N, 87.94372° W). One gram of soil was serially diluted in sterile water, plated on a Luria-Bertani agar (LBA), and incubated at 28 °C for 48 hours. Isolated bacteria were picked and patched to LBA containing cycloheximide to create library plates for additional experiments. The isolates were screened for antibiotic activity using LBA, Potato Dextrose agar (PDA), Tryptic Soy agar (TSA), and 10% TSA plates containing either Bacillus subtilis (B. subtilis) or Escherichia coli (E.coli). When conducting a gram stain, the bacteria was found to be gram-positive bacillus. PCR amplification of the DNA sequence for the 16S rRNA gene was attempted, but unsuccessful. The bacteria will continue to be studied for the possibility of future pharmaceutical therapeutics.

83 Searching for Heroes in Unlikely Places: The Search for Antibiotic-Producing Bacteria in Soil

KYRIE STAAB
University of Wisconsin - Whitewater

Evolution has produced bacteria that can produce antimicrobial substances that kill other bacteria, which helps these bacteria compete for resources in nutrient-poor environments. Unfortunately, the same evolutionary pressures that give rise to antibiotic-producing bacteria, also give rise to bacterial antibiotic resistance. While antibiotic-resistant bacterial diseases are harder to treat and result in more deaths, there are many ways to combat the challenge of antimicrobial resistance, including the discovery of new antibiotics. In cooperation with the Tiny Earth Project, soil samples were collected from areas in southern Wisconsin, and bacterial isolates were purified and screened for antibiotic production to aid the search for novel antibiotics. Preliminary results indicate that there are two antibiotic-producing bacterial strains from two separate soil samples that can inhibit at least two types of bacteria. Further studies are ongoing to identify the genus of the isolates.

84 Tiny Earth

KATRINA SVETLICHNYY, EMMA MLECZKO
Northeast Wisconsin Technical College

Tiny Earth is a research project that strives to find new antibiotics in our very own soil around us. As the supply of effective antibiotics has dramatically dropped in recent years, as students study microbes from local soils, they are learning evidence-based hands-on material through interactive research. Our project's purpose is to identify microbes releasing antibiotics. We collected our soil samples from a twenty-year-old well-maintained garden and compared it to a park where the only maintenance is mowing. Comparing a tendered garden with an unkempt park we believed that the garden and park will have lots of bacteria that would differ from each other. In lab we gathered the soil and followed that with diluting the soil in order to grow the bacteria, once we did that we changed the growing environments and followed that with testing it against relatives of different ESKAPE pathogens, in our current step of researching, we are doing polymerase chain reaction testing which runs our bacteria in a vigorous cycle to confirm the 16s rRNA gene is present. Our research is still progressing, and we will be following it with gel electrophoresis testing. Research so far has shown that the garden soil sample showed antibiotic activity against Acinetobacter and Bacillus while the park soil sample tested against S. epidermidis and only a little bit of activity against Acinetobacter. With the research we have thus far, we can conclude that our soil microbes are active because they have worked against more than one relative of ESKAPE pathogen. As we continue our research, we will be able to figure out specifics on our bacteria.

85 The Search for New Antibiotic Producing Bacteria in Green Bay

LEE LEMIRAND, JACOB MANNENBACH
Northeast Wisconsin Technical College

The purpose of the Tiny Earth Project is to find new antibiotic-producing bacteria from soil, contributing to the fight against antibiotic resistance. This is an increasing threat in our world as more pathogens become resistant to existing antibiotics. We took soil samples from two different community gardens in hopes of finding a rich environment for bacteria to grow. Our primary objective in taking from two gardens (Green Bay Community Church in Howard, WI and 5th Street Community Garden in Green Bay, WI) was to create a wide scope from which to get antibiotic-producing microorganisms. We believed we would not find new antibiotic-producing bacteria due to it being such a rare occurrence to find those bacteria. We narrowed down and tested our most promising bacteria against several relatives of ESKAPE pathogens (organisms with high antibiotic resistance) for antibiotic activity against these ESKAPE pathogens. One sample yielded antibiotic activity against Bacillus, a relative of the ESKAPE pathogens and our testing for confirmation on new antibiotic activity is ongoing at this point.

86 Foggy Soil

NENG XIONG
Northeast Wisconsin Technical College

In America, many people are becoming infected with multiple antibiotic resistance bacteria that is why this research is vital. In Hobart, Wisconsin, it is very misty on both sides of the street till mid-morning. I hypothesized that the fog caused a moister environment to allow better bacteria to grow. I collected the soil and performed a soil dilution test. After trying multiple conditions, I recorded how they grew. I chose 20 colonies for and tested them against *Erwinia* and *S. epidermidis*. The *S. epidermidis* plate had three producers and *Erwinia* had none. I chose a single bacterium to continue with and tested it against ESKAPE pathogens and found additional activity against B. subtilis. More experiments will happen to identify this bacterium.

87 Antibiotic-Producing Freshwater Fungi in the GB Area

MORGAN FIMREITE
St. Norbert College

To compete with other microorganisms in crowded biological environments, fungi produce certain chemical compounds called secondary metabolites. These compounds are natural products that are not necessary for an organism's essential life processes. It is possible that these secreted compounds have the potential to serve as antifungal drugs. Many of the current available antifungals disrupt cell membranes or walls. The lack of variety of antifungal agents is problematic when attempting to treat a human fungal infection. As the number of drug-resistant microorganisms increases, this creates a challenge for the medical and pharmaceutical fields. If fungi have developed resistance to antifungals, there is little hope of being able to kill them with other currently available antifungal drugs. The contamination of waters with antibiotics and fertilizer runoff has been shown to change microorganisms and possibly allow them to develop more antibacterial properties. We investigated several bodies of freshwater including the Fox River, Abbey Pond, Bower's Creek, and Lilly Lake for microbes living near the sediment. We plated samples on LB and Potato Dextrose plates in hopes of encouraging the growth of both bacteria and fungi. After isolating visually different microbes, we competed with several ESKAPE pathogens. We plan to sequence promising antibiotic-producers to identify them and further study the secondary metabolites they secrete. We plan to isolate and purify these metabolites using extraction and chromatography techniques. NMR, IR, and UV spectroscopy as well as Mass-Spectroscopy analysis will be conducted to learn more about their chemical structure and composition.

88 Tiny Earth

STACEY JOHNSON
Northeast Wisconsin Technical College

It has been found that with the increase of population of the United States and the rise of infection rate there are more and more antibiotic-resistant infections causing mortality. There are not enough new antibiotics being discovered to keep up with this resistance trend. Tiny Earth has the goal of educating students and the public about the soil crisis. They have predicted that the soil is eroding quickly while projecting it to be entirely depleted by the end of the 21st century. Being that the soil is a big source of antibiotic discovery there will be less chances to discover new novel antibiotics. By educating students about this crisis and using the potential discovery of new antibiotics to teach students about the process of laboratory research. As a student we were posed with the task to obtain a soil sample from an area we thought would produce a novel antibiotic. I had chosen one of the horse turnout areas for my collection site thinking that there would be a lot of bacteria present in this soil. We diluted and plated our soil from our chosen sample using the streak plate technique. The microbes found were inoculated and screened against the bacteria *Staphylococcus epidermidis* and *Erwinia carotovora* to see which microbes produce antibiotics against the bacteria. I had four clear zones of inhibition found on the E. carotovora plate, and five zones observed on the *S. epidermidis* plate and no dual producers. We then chose the producer that we thought to give us the best chance of being an antibiotic producer. We moved forward with this sample and screened it against the ESKAPE pathogens. My results showed two good possibilities of an antibiotic producer. My hypothesis was not accurate as I had predicted finding twenty antibiotic producers. We are still moving forward with research in finding a novel anti

89 Digging for Solutions: Unearthing Antibiotic Producers

BENJAMIN DENAMUR, NICOLAS STRONG
University of Wisconsin - Green Bay

Antibiotic resistance is a looming global health crisis, creating an urgent need for innovative antibiotics. Tiny Earth research aims to identify antibiotic-producing bacteria in soil samples to help address this problem. Soil for this research was collected near Sugar Creek (44.787736, -87.659639) in Southern Door County, an active creek environment expected to harbor diverse soil isolates. A one-gram soil sample was serially diluted and plated on Luria-Bertani Agar (LBA) containing cycloheximide to isolate soil bacteria. Master plates were created on LBA with cycloheximide and Potato Dextrose Agar (PDA) with cycloheximide. Soil isolates were then patched to PDA with cycloheximide, LB agar with cycloheximide, 10% Tryptic Soy Agar (TSA), and 100% TSA, and inoculated with Escherichia coli (E. coli) or Bacillus subtilis (B. subtilis) screen for antibiotic activity. Of the nine isolated strains, six displayed antibiotic activity. DNA for the 16S rRNA gene was amplified by polymerase chain reaction (PCR), sequenced, and subjected to Basic Local Alignment Search Tool (BLAST) analysis to determine the identity of each antibiotic producer. Of the six, four were identified through PCR sequencing and BLAST analysis. This research contributes to the discovery of potential new antibiotics and provides valuable hands-on experience in antibiotic research for students. Tiny Earth's efforts in soil microbiology education address the growing public health challenge of antibiotic resistance by engaging students in meaningful, real-world research.

90 Cattle soil producing antibiotics

ELLA KILLINGER
Northeast Wisconsin Technical College

Since the 1950's we have been faced with the issue of antibiotic resistance. Antibiotics are becoming less and less effective due to antibiotic resistant pathogens becoming more prevalent in society and our everyday lives. This issue is predicted to cause diseases like the simple infection, strep throat, or STI's, to lead to mass amounts of deaths by the year 2035; as well as we are already seeing many deaths due to antibiotic resistance. The Tiny Earth organization has given us the opportunity to use soil and find new microbes that could produce antibiotics in hopes to be able to help with this life-threatening issue. With this we can present our findings at a conference to get our communities involved with our findings. I decided to take soil from the pasture my cattle live in. My hypothesis was that there would be antibiotic producing microbes here due to the cattle living off this land and their natural waste going back into this land daily. To test the soil, I diluted and plated it using the streak plate technique. The microbes I found were then inoculated and screened against the bacteria *S. epidermidis* to see if antibiotic producing microbes were present against those bacteria. I found that one of my microbes produced antibiotics against *S. epidermidis*. I then tested my microbes against the ESKAPE pathogens. This showed that my microbe did not have activity against the ESKAPE pathogens. My hypothesis was accurate, as I did have an antibiotic producing microbe in my soil. My research is still ongoing, so some results are yet to be determined.

91 Bone-biotics

KADIN BECKER
Northeast Wisconsin Technical College

For more than thirty years, the research for newly sourced antibiotics has been completely neglected. The rapid rate of antibiotic-resistant microbes combined with the lack of financial benefit for larger companies and organizations has led to an almost complete halt in the search for new antibiotics that could save countless lives. Due to this, the Tiny Earth Organization has allowed students to collect, identify and hopefully discover the next lifesaving antibiotic-producing microbe. I was eager for the opportunity to partake in the Tiny Earth project and spent some time contemplating what might contain effective microbes that other students wouldn't have access to. Growing up on a dairy farm, I knew of a very specific spot in our woods where the previous farmer appeared to have disposed of his dead cattle. This pile was clearly quite old, and many bones were deep into the decomposition phase. My hypothesis was that these bones would be successful in producing antibiotics. After diluting the sample to hopefully isolate the bacteria, I inoculated several plates with the mixture to see what conditions it grows best in. The next test I did involve testing my colonies against Erwinia and S.epidermidis. I found that six out of my twenty selected colonies showed positive results. Next, I chose a single double producer from the previous test and applied it to eight pathogens. This resulted in positive results for two more, E.coli and Enterobacter. This fully supports my hypothesis that my sample would be effective. Further testing will help in the identity of my soil bacteria.

92 Discovering Antibiotic-Producing Microbes in Soil

JESSICA OLEJNICZAK, JENNIFER POLKINGHORNE
University of Wisconsin - Green Bay

The rise of antibiotic-resistance has become a major public health concern, causing increased mortality rates and difficulty treating infections. In order to lessen the burden of healthcare workers and increase the quality of life among those with hard-to-treat infections, there is a growing need to research new antibiotics. The Tiny Earth research project was developed for students to discover antibiotic-producing bacteria in the soil that can help with this worldwide crisis we are facing. One gram of soil was collected on the University of Wisconsin – Green Bay campus and then serially diluted and placed on four different LB plates with cycloheximide, to eliminate the possibility of fungal growth and enhance the isolation of prokaryotes. Master plates were then created by picking and patching the isolated bacteria using a grid layout to place 12 single colonies. LB and PDA were successful in growing 11 out of 12 colonies patched. The bacteria that had successful growth were screened for antibiotic activity by inoculating them on plates containing either Escherichia coli (E. coli) or Bacillus Subtilis (B. subtilis). Three total isolates were positive for antibiotic activity, all on the B. subtilis plate. The three isolates then underwent PCR, amplifying the DNA for the 16S rRNA gene, allowing BLAST analysis to determine the identity of each bacterium. Finally, various biochemical tests were performed to provide more information on the diversity of our samples. This research is important, as it could lead to a new antibiotic discovery.

93 *Citrobacter freundii* and *Bacillus subtilis* as Potential Antibiotic Producing Soil Organisms

EMMA HERB, MEGAN PIERCE
St. Norbert College

As antibiotic resistance continues to spread, the efforts to find novel antibiotics have increased to defend against bacterial pathogens. In association with the Tiny Earth Project, a soil sample was obtained from a flower bed in Ashwaubenon, WI with the goal of isolating potential antibiotic-producing microorganisms. 13 different organisms were isolated using a series of serial dilutions and plated onto 1/10 CYE and 1/10 TS agar media plates. Further experimentation included plating these organisms against common ESKAPE pathogen safe relatives. Three of the original isolated colonies were shown to exhibit antimicrobial behaviors against the ESKAPE pathogen safe relative Bacillus megaterium. Additionally, one isolated colony exhibited antimicrobial properties against the ESKAPE pathogen safe relative Enterobacter aerogenes. To further analyze these colonies, a series of PCR amplification and cleanup measures were performed using known 16 S ribosomal RNA primers (63F and 1387R). The sequencing data obtained was then submitted to BLAST and compared to the sequences of known organisms. Further identification of the unknown isolated organisms was recognized as Citrobacter freundii and Bacillus subtilis. With ongoing experimentation and testing, these species can be further analyzed for their potential to express antimicrobial properties.

94 Soil Analysis from a Water Run Off on St. Norbert College Campus

PAIGE DIERCKS
Northeast Wisconsin Technical College

With the constant growth of antibiotic-resistant pathogenic bacteria, there is a need for new antibiotics to fight these diseases. As a part of the Tiny Earth Project, bacteria were isolated from soil collected from a water runoff area between apartment buildings. They were cultured on 1/10 TSY and 1/10 CYE agar media. Eight colonies were patched onto the lawn of two safe relatives of an ESKAPE pathogen to find if any of them exhibited antimicrobial properties. The safe relative that was used was Acinetobacter baylyi. Of the eight, five exhibited antimicrobial zones. PCR and sequencing of the 16s rRNA gene of each bacteria was done to determine the species of bacteria. Of the five selected for PCR, three were successful and the 16S rRNA gene was sequenced. Within the three that were successful, one sequence gave solid sequencing results. These data showed it was a strain of Flavobacterium. The other two samples did not provide solid sequencing on what it could be. Sequencing from one of the forward primers gave it could be Brevundimonas or Mycoplana strains. The reverse primer gave inconclusive results due to needing more templates. With sample B, the forward primer given could be Shewanella. The reverse primer said it could be a Pantoea strain. Future biochemical testing is needed to determine the strains of bacteria fully.

95 Bioorganic Research and Development of Antimicrobial Agents

JUSTIN BENOIT, HUNTER HUCEK
St. Norbert College

Antibiotic resistance is an increasing global problem. The Tiny Earth program is dedicated not only to reducing the danger of antibiotic-resistant bacteria by leveraging the work of the many microbiology-based undergraduate courses running every year, but also using the search for solutions as a means to provide authentic research experiences to undergraduates on a large scale. One approach to addressing this crisis is adding new potential antibiotics by the discovery of antibiotic producing bacteria, which are common in soil. Another is efforts to reduce infections using disinfectants and antiseptics. The Bioorganic Research and Development course at St. Norbert College aims to use Tiny Earth methods and ideas to conduct authentic research into the actions of antimicrobial agents associated with industrial applications. Surfactants and organic acids used in disinfecting wipes produced by Rockline Industries, along with novel indoles, were synthesized by students in an organic chemistry lab and tested for antimicrobial action in a microbiology lab by groups of students over the course of a semester. Results of this interdisciplinary work will be presented.

STUDENT POSTERS

96 *Pseudomonas* Population in Soil from Menomonee Falls

JOSEPH WALLOCH, MADISON ARAUJO
St. Norbert College

Naturally occurring bacteria can be used as a means to study antibiotic resistance against medically relevant microorganisms. A sample of an antibiotic-resistant bacteria was collected from soil in Menomonee Falls, Wisconsin. With the support of the Tiny Earth Project, we were able to subsequently plate the bacteria on 1/10 TS AGAR media to test for antibiotic-producing microorganisms. A total of 20 different organisms were patched along with safe relatives of ESKAPE pathogens. 4 of the colonies that were patched produced antibiotic activity. During the plating stage, each of the four colonies produced antibiotic activity and showed varying morphologies while on the plate. The 4 colonies were gram negative and were rods. DNA was extracted from the 4 colonies and DNA purification and amplification were conducted. The 16S rRNA gene was sequenced and it was determined that all 4 colonies belong to the genus of *Pseudomonas*. Future analysis will include various biochemical testing on each colony to specify the distinct species of *Pseudomonas*.

97 Unearthing Antibiotic Potential: Exploring Soil Microbes from Cecotropes to Indoor Plants

GLORIA KOHRMANN, DAYNA BLOHOWIAK
St. Norbert College

Life on Earth has evolved from the Paleozoic to the Cenozoic era, bringing human health challenges that current antibiotics cannot easily address. To aid in the search for antibiotic- producing microorganisms, soil samples were collected from a span of sources ranging from cecotropes to indoor plants, showing that evolution occurs even in mundane environments. In partnering with the Tiny Earth Project, we first examined bacteria (1.24 x 10^3 CFU/mL) in cecotropes, which showed absence of antibiotic production when patched in lawns of safe relatives of the ESKAPE pathogens. In another study, bacteria from a common household plant demonstrated some antibiotic properties when patched in lawns of ESKAPE pathogens. This colony was one of many that exhibited antibiotic properties in all the ESKAPE lawns that were plated; observed as a zone of inhibition. The bacterium's morphology on the patch plate showed a dull, dry, moderate, black, filamentous colony. However, once streaked for isolation the morphology showed a moderate, glistening, non-pigmented, transparent, filamentous colony. Gram staining revealed gram-negative rods, and 16S rRNA amplification with universal primers 63F and 1387R produced a 1.3 kbp PCR product. After PCR clean-up and nanodrop validation, samples were prepared for sequencing and sent out for analysis. Future steps include evaluating sequencing, running the sequence through BLAST to retrieve comparable genome sequences, then eliminating and identifying organisms who's sequencing best match that of the product analyzed from the indoor houseplant soil sample. Results will be presented.

98 Could Ducks Aid in Antibiotic Resistance?

MADDELYN OLIVE
Northeast Wisconsin Technical College

The healthcare system is struggling due to antibiotic resistance. With increasing difficulty to treat infections, patients are suffering from longer hospital stays and inadequate care. The Tiny Earth organization recognized this issue and constructed a way to help solve this crisis. Compiling over 200 microbiology students every year to collect soil from areas all around Wisconsin is an effective and efficient way to make change quicker. To add some diversity into my search for novel antibiotics, I chose to collect soil from my duck pen at my house. My hypothesis was that I would find at least one novel antibiotic in my soil collection, due to my pet ducks trafficking around various microbes from my yard and the creek behind my house. After obtaining an adequate soil collection, I diluted and plated the soil following the streak plate method and left it to be inoculated overnight. I then carefully chose 20 individual colonies off my plate and tested them against the bacteria Staphylococcus epidermidis and Erwinia carotovora. After testing for antibodies against the bacteria, I had 5 colonies that displayed antibiotics. One of my colonies was a double producer so I continued with it for the remainder of the search, testing it against several other forms of bacteria. I am currently unable to determine if my hypothesis is accurate yet without doing more testing on my chosen microbe. My research on my backyard antibiotic producing soil is still in process, with hopes to determine if my microbe is a suitable antibiotic.

99 Antimicrobial Producers Found in Farm Field Soil

GABRIELLE BRANDT, CASSIDY BURKE
St. Norbert College

Due to the growing concern surrounding antibiotic resistance, we decided that testing for naturally occurring antibiotics in soil may be beneficial. In collaboration with the Tiny Earth Project, bacteria were isolated from a soil sample taken from a farm field in Reedsville, WI. These bacteria were cultured on 1/10 TS and 1/10 LB agar media. To detect possible antibiotic producing bacteria, colonies from a spread plate were used to create a patch plate containing 10 isolated colonies. This patch plate was replicated on a lawn of the ESKAPE pathogen safe relative, Bacillus megaterium. Of the 10 colonies on this plate, 2 were observed to be producing antimicrobial properties against the pathogen in the lawn. The first antibiotic producing colony was cultured on 1/10 TS and is a gram-positive rod that is light orange in color. The second antibiotic producing colony was cultured on 1/10 LB and is a gram-negative rod that appears a creamy-white color and mucous-like in texture. Colony PCR was completed and following that, sequencing of the 16S rRNA gene was performed. The results indicated that the two bacteria that have been cultured are a Fictibacillus and a Lysobacter. Future directions include a series of biochemical tests to further determine the species of these unknown bacteria.

100 Exploring Microbial Diversity at Pine Acres Golf Course

GRACE LEMORANDE
Northeast Wisconsin Technical College

In the 1940's, the first antibiotics became available to the public. This was a revolutionary time for medicine. Before antibiotics, bacterial infections were the leading cause of infection and ultimately, death. Unfortunately, signs of antibiotic resistance appeared only a decade after they became available to the public. For years scientists have worked to fight antibiotic resistance, with the help of the Tiny Earth Organization, students are able to analyze soil from around the community in hopes to discover new antibiotic producing microbes and present their findings to scientists and the community. To incorporate my community in the project, I chose to collect soil from a golf course local to me, Pine Acres Golf Course in Abrams, Wisconsin. I hypothesize that I would discover antibiotic producing microbes because microbes tend to thrive in warm and moist environments, such as the soil from a golf course. Golf courses offer a favorable environment for microbes to multiply. I gathered the soil sample, then diluted it to get isolated colonies on a plate. Then I tested the sample up against two bacteria, *Erwinia* and *S. epidermidis* to see if any sample released any antibiotics. The sample had one potential antibiotic producer on the *S. epidermidis* plate which was further against the ESKAPE pathogens. Although the results are still pending, my hypothesis was correct, I did discover one antibiotic producing microbe.

101 Antibiotic Producing Microbes in Pond Water and Bamboo Plant Soil

JOREN KILSDONK, MORGAN FIMREITE
St. Norbert College

Microorganisms, namely bacteria and fungi, often produce antibiotic agents as a means of competing with other nearby microorganisms. Finding and cataloging these antibiotic agents can be a fruitful endeavor both for intellectual and medicinal purposes, especially as lack of antibiotics is a major clinical problem. We collected samples from places that we thought would be home to many different bacterial species which might promote bacterial competition. These include samples from potted bamboo soil and sediment from a local pond. After diluting original samples and plating on diluted TSY and CYE media as well as PDA, colonies were selected and isolated. Bacterial lawns were created with the safe relatives of six ESKAPE pathogens and collected microbes screened against the safe relatives to observe for growth inhibition. The gram staining status and visual characteristics of microbes that showed antibacterial abilities were observed by microscopy. The 16S ribosomal gene of these microbes was amplified, sequenced, and put into GenBank's BLAST to identify them before screening them for antibiotic properties, such as the secondary metabolites they produce. We hope to present our results from this project, including any secondary metabolites of interest that may be clinically helpful.

102 Bacteria Isolates from Garden Soil in Wrightstown, WI

MEGHAN RIHA, ZOE DRIVER
St. Norbert College

As the number of bacteria resistant to antibiotics continues to increase, there is a scientific push for finding new ways to combat this problem. One potential solution is finding novel bacteria that produce antibiotics. In partnership with the Tiny Earth Project, bacteria were isolated from soil found in a small outdoor garden in Wrightstown, WI and cultured on 1/10 LB and 1/10 TS agar plates. Using ESKAPE safe relatives Escherichia coli, Staphylococcus epidermidis, Acinetobacter baylyi and Enterobacter aerogenes the 48 isolated colonies were tested to see if they had any antibiotic producing properties. Four different colonies were perceived to be "fighting" off the pathogen meaning they were producing some type of antibiotic. Each of the bacteria types had different characteristics including one colony yellow in color with a film-like appearance, another one dark purple and globular-shaped, a yellow circular colony with a darker edge, and the last colony sheer white in color with undulated edges. To find the identity of each specific colony PCR and sequencing of the 16S rRNA gene were conducted. The sequences were entered into BLAST and the results matched with Flavobacterium sp. strain, Massilia sp., and Acinetobacter sp. exhibiting three of the four colonies tested. Unfortunately, one of the sequences was unable to be read. In future studies, further investigation will be taken to find the unidentified colony.

103 Antibiotics and Animal Activity

TIONNA WAYKA, NATE BECKER, DALTON PANSKE, STEEL TORREZ
Northeast Wisconsin Techncial College

The Tiny Earth Project allows students to enter into the conversation of antibiotic resistance, an issue that has troubled scientists, healthcare, and the general population for decades. The increase of antibiotic-resistant pathogens and the shortage of new antibiotics has become a prominent issue in the world of medical science. The Tiny Earth Project provides students with an opportunity to test the soil in chosen environments in attempts to find and culture a new antibiotic-producing bacteria. Our group has chosen to compare soils presenting with different levels of animal activity and moisture. Our locations included a private residence, a public park, a community pond, and a privately owned chicken coup. We hypothesized that soils with more animal activity and moisture would provide a more suitable environment for antibiotic-producing bacteria to grow, due to the diverse selection of nutrients. Through dilution we were able to isolate colonies of bacteria found in our soil and test them. At least one possible antibiotic-producing bacteria was found in all four samples when testing the bacteria against Erwinia and S. epidermidis. After choosing one possible antibiotic-producer, bacteria were tested against the eight relatives of the ESKAPE pathogens. The bacteria from the chicken coup reacted against B. subtilis and E. coli while the other three soil bacteria only reacted against B. subtilis. Our hypothesis regarding animal activity seems to prove true. At this point, there are still stages of testing and observing to be done, so our results are not yet final.

104 Dirt: Enemy or Solution

SUSANA CALZADA, JENNIFER ELGIN
Northeast Wisconsin Technical College

Finding effective antibiotics has become more difficult to find in today's world. We have been given the opportunity to work with the Tiny Earth project to research diverse types of soil and to see if there are any microbes that produce antibiotics. Our team had the opportunity to research two distinct types of soil to find diverse microbes. Our team obtained samples from private back yards. One soil sample is a well mix of organic matter that is nutrient dense (residential), while the second has a clay, low organic matter medium(rural). Our team hypothesized that we would find more microbes in the residential soil over the rural area because of the amount of organic matter vs nutrient availability. This in turn would give us a higher opportunity to find microbes that produce antibiotics. By using Tiny Earth methods, from the collection of the samples, growing microbes on different media plates, and the use of safe pathogens, our team compared the microbes grown at NWTC's lab with the microbes that Tiny Earth is researching for these antibiotics. This indeed shows that the residential media grew many more colonies of bacteria, but not necessarily ones that produce antibiotics. Here are the results and processes that show our success and failures of the study.

105 The Future of Antibiotics on Beef Versus Dairy Farms

KYRA JORDAN, CASSADY NITZ
Northeast Wisconsin Technical College

Since antibiotics started being discovered bacteria have been mutating to become resistant to our antibiotics and we have been fighting a losing battle. The main issue is when people are prescribed antibiotics, they are not finishing them, and this allows the bacteria to come back stronger than before. Scientists have stopped looking for new antibiotics due to the excessive cost of doing so, and that is where we come in. Through Northeast Wisconsin Technical College, we were able to partner with the Tiny Earth Organization to help combat the issue of the antibiotic shortage using our families' farms. We decided to compare soil from a dairy farm and beef farm. We thought that looking at our farms' soil that they would have a wide array of bacteria. After collecting our soil, we diluted it and grew it on a plate. We then assessed the microbes against the Erwinia and S. epidermidis bacteria to see if any of the microbes would produce antibiotics against those bacteria. The beef farm had three antibiotic producing microbes and the dairy farm had one. We each choose one bacteria from our plates to test against the ESKAPE pathogens. Both the dairy and beef farm antibiotic that was tested positive against the Bacillus bacteria. Our hypothesis was that the dairy farm would produce more antibiotic microbes than the beef farm, but we were incorrect. Our soil is still being evaluated and therefore we are still waiting on some results.

106 Exploration of Eendophytic Species from Wisconsin Towards the Discovery of New Natural Product Antibiotic Scaffolds

SARAH WELSCH
St. Norbert College

I have spent the fall semester studying endophytic organisms found within plants native to the Wisconsin area for their ability to produce antibiotics. An endophyte is a type of bacteria, fungi, or microorganism that colonizes the interior of plants without causing harm to the plant. The discovery of a novel endophyte within a plant could lead to a new type of antibiotic treatment that bacteria have not encountered before. This is significant especially in today's era with bacteria becoming more resistant to our current antibiotic methods, rendering our treatments close to useless. We selected plants that have been used historically for medicinal tasks. This semester, we were able to collect seven different endophytes from the plants: Sweet Flag, Blackberry, and Grey Alder bark. Once the endophytes were isolated, we tested them against safe, ESKAPE pathogen relatives to see if the endophytes can produce molecules that can slow or prevent the growth of the pathogenic bacteria. We used a strain of Escherichia coli and Enterobacter aerogenes pathogens and are currently testing more. We are in the process of using 16S rRNA sequencing to identify our endophytes that have shown antibiotic activity. Novel endophytes will be cultured on a large scale to extract their secondary metabolites. Isolation of the active compound(s) will be accomplished using extraction and chromatography techniques. To determine the chemical structure of the compound, we will use NMR, IR and UV spectroscopy.

STUDENT POSTERS

107 Antibiotics and Us

TRAVIS MARSHALL
Northeast Wisconsin Technical College

Since antibiotics were first discovered, in less than 100 years, we have experienced a growing concern for antibiotic resistant bacteria. Globally, at least 1.27 million people died from antibiotic-resistant infections in 2019. Things we can do as future healthcare providers is inform the public on how vital finding new antibiotics is and how to prevent antibiotic resistance for future generations. We started our journey, of potentially finding a new antibiotic, by gathering a soil sample. I took my sample close to my apartment as I live on the outskirts of Green Bay where deer, turkeys, cranes, and other wildlife roam. My hypothesis was that taking a soil sample from such a diverse ecosystem, it would allow me a better chance to find a bacteria that produces an antibiotic that has yet to be discovered. We started by diluting our soil samples in tubes filled with phosphate-buffered saline. Next, we selected 20 different individual colonies that grew on our LB plates. Of the 20 selected individual colonies, we screened them against *Erwinia* and *Staphylococcus epidermidis* bacteria to see if our bacteria would produce any antibiotics against them. Of the 20 selected colonies, only 2 produced activities against *Erwinia*. I then took one of my antibiotics producing colonies and screened them against 8 other pathogens. Between 8 of the pathogens we screened against, my bacteria had activity against 2 additional pathogens. As we continue with our study, I am hopeful that my bacteria will get selected to go on for further testing and can lead to the discovery of a new antibiotic.

108 Meadows In Motion to Discover Wonder Drug

MORGAN MALECKI, LEILA GARCIA
Northeast Wisconsin Technical College

Antimicrobial resistance is currently one of the top threats to public health not only in the United States but globally. The mortality rate over the last decade from untreatable infectious diseases has amplified. The Tiny Earth Project allows us to try and uncover new microbes within our soil that could potentially produce antibiotics. For this project, we decided to collect soil from our family farms. One of the farms is an inorganic dairy farm, and the other is an organic dairy farm. We hypothesized that the soil from the organic dairy farm would have a larger amount of microbial diversity than the inorganic dairy farm. To prove our hypothesis, we conducted several different experiments using multiple methods. Soil dilution and the streak plate technique were used to allow for growth, cultivation, and single colonies of our bacteria. The microbes we were able to grow were then inoculated and tested against the bacteria Erwinia and S. epidermidis. Both the inorganic and organic dairy farm samples only had one microbe that would possibly lead to an antibiotic releaser. Then we tested our one microbe against the relatives of the ESKAPE pathogens. Our results showed only one antibiotic producing microbe for both the inorganic and organic dairy farm samples. Therefore, our hypothesis was incorrect but our experiments are still underway and there are more results to come.

109 Researching Soil Bacteria to Help Solve the Worldwide Antibiotic Crisis

ALISHA JAHNKE, MITCH STARRY
University of Wisconsin - Green Bay

In the world today, numerous strains of bacteria are becoming resistant to antibiotics due to the overuse of medication in farming and healthcare. Tiny Earth research aims to tackle this issue by studying antibiotic-producing bacteria in soil samples. A soil sample was collected near the bank of the Waupaca River at 44.366°N, -89.123°W at a depth of 2.5 inches. One gram of soil was serially diluted in sterile water and plated on Luria-Bertani agar (LBA), containing cycloheximide to deter fungal growth. Isolates were patched onto potato dextrose agar (PDA) and LBA master plates. Of the 12 isolates picked, four expressed antibiotic activity against Escherichia coli (E. coli) and Bacillus subtilis (B.subtilis) and were numbered 1-4. Isolate 2 expressed antibiotic activity on LBA and 100% tryptic soy agar (TSA) against E. coli and B.subtilis regardless of cultivation media. Isolates 1, 3, and 4 expressed activity against B. subtilis on all cultivation media. Further characterization via BLAST analysis of the DNA sequence for the 16S rRNA gene and gram-stain analysis showed that all isolates were of the Pseudomonas genus. Additional experimentation was performed using biochemical tests to confirm the identity of the antibiotic producers. Tiny Earth research presents a unique opportunity to students to aid in the antibiotic crisis and to gain experience in a lab setting for future careers. If this project continues, eventually new antibiotics will almost certainly result.

110 Digging Deep: The Search for Antibiotic Potential in Soil

EMMA SCHEPP, LAUREN BORCHARDT
St. Norbert College

The goal of our investigation is to isolate antibiotic-producing organisms from the environment, which can help minimize diseases caused by ESKAPE pathogens. These organisms are naturally occurring and can work therapeutically. We worked with the Tiny Earth Project to isolate bacteria (8.8x105 CFU/mL) from soil near train tracks in De Pere, Wisconsin. We cultured two samples on 1/10 casitone-yeast extract (CYE) agar media and two on 1/10 lysogeny broth (LB) agar media. 24 colonies from the four isolation plates were patched onto each lawn of the following ESKAPE pathogens: *Pseudomonas putida*, *Acinetobacter baylyi*, *Bacillus megaterium*, *Staphylococcus epidermidis*, and *Escherichia coli* to reveal any antibiotic-producing colonies. Five colonies were identified that manifested antimicrobial activity against the ESKAPE PATHOGEN lawn. Three isolated colonies with potential antibiotic resistance traits were identified on the *Bacillus megaterium* lawn. All of which were gram + *bacillus*, creamy, and beige strains. They all had a shiny, mucous appearance. Isolate #1 also showed endospore-forming characteristics. The fourth colony on the *Staphylococcus epidermidis* appeared to be yellow with a transparent outer ring and was a gram-negative rod. Another colony from the *Escherichia coli* plate was a gram-negative coccus and appeared as a raised, translucent white colony on the plate. Several biochemical tests were performed to understand the metabolic processes of the isolates. PCR and sequencing of the 16S rRNA gene were performed on the bacterial isolates.

111 Chicken Coop Soil

SAMANTHA GRAVES
Northeast Wisconsin Technical College

Long ago, bacteria took millions of lives through bacterial infections with little to no effective treatments available. In the 20th century, scientists discovered the first mass-produced antibiotic from a piece of molded fruit. Little did we know that antibiotics would not cure or treat viral infections, and that they needed to be taken appropriately to prevent the bacteria from mutating and creating resistant strains. Through Tiny Earth, we are looking to find novel antibiotics from soil-borne bacteria to face this resistance crisis we have created. I collected my soil sample from my garden where my chickens roam and the apple trees grow. I predict that I will have many microbes growing in my soil and have a chance at finding a novel antibiotic. I felt this location was nutrient rich for microbes. I then diluted the soil and 20 colonies were placed on an Erwina carotovora or *Staphylococcus epidermidis* plate to see if any more zones of inhibition would appear. I had 3 different sites with zones of inhibition of similar sizes and 2 of those were double producers. From there, nine different bacteria were divided onto a plate and I chose a colony off my master plate to place on to each of these bacteria to test further for zones of inhibition. The results from this were no zones of inhibition were noted to any of the 9 bacteria. My soil is rich with microbes but may not be hosting a novel antibiotic producer at this time.

112 Antibiotic Producers from NWTC Soil Collection

MICHAELA STICH
Northeast Wisconsin Technical College

For a long time now, scientists and members of the medical community have worried about antibiotic-resistant pathogens and the danger that they pose to humans. This dilemma has had t school, so direct negative implications on our society's health and even contributed to mortality rates. The Tiny Earth organization and NWTC have given me and other students the chance to try to find new antibiotic producing microbes. We also have been given the opportunity to present our findings to the local community during a public conference. I chose to collect my soil from NWTC grounds, and in the lab, I have been conducting multiple tests on my collection. My hypothesis was that the NWTC soil bacteria would multiply without hesitation because of how much foot activity it has and is exposed to. I diluted my soil sample and plated it on agar plates using the streak technique. The microbes that I found in my collection was then screened against *S. epidermidis* and *Erwinia* in hopes that my bacteria would produce antibiotics against them. Upon the completion of that experiment, I found that my bacteria was not a double producer however it did show activity against *S. epidermidis*. While I did have 6 options of producers to choose from, I only picked one to continue with and test against ESKAPE pathogens. My hypothesis was not correct, as I expected there to be more antibiotic activity than there was. My testing and research on the soil collection is still ongoing in the lab at further results are still to be determined.

113 Exploring Possibilities: Antibiotic Producing Microbes Near Point Beach Nuclear Plant

LYDIA SCHMIDT
Northeast Wisconsin Technical College

Antibiotic resistant pathogens are an unseen threat to our health, yet their impact is significant. They pose danger to not only our wellbeing but the future of medicine. Which is why at NWTC we are doing the Tiny Earth lab to discover antibiotic containing bacteria. Bacteria can be found in numerous environments, so why not start with the ground beneath our feet. I chose to take soil from near Point Beach Nuclear Plant in Manitowoc Wisconsin. I chose this location because soil near a nuclear plant may exhibit different characteristics than the surrounding areas and hypothesized that

I would find three antibiotic containing bacteria. After collecting, I brought my dirt to NWTC where we continued our experiment; diluting, growing on different mediums, in different conditions, screening for antibiotic production. When screening, we first tested against S. epidermidis and Erwinia. Out of the twenty microbes tested two were active against S. epidermidis. Next, I tested one microbe against the ESKAPE pathogens, and no activity was found. This had countered my hypothesis of finding three antibiotic producers in the soil I had taken. Further testing is still being conducted and more results are yet to come.

114 Off the Beaten Path

BROOKLYN THOMPSON, KAYLA BILLICK
Northeast Wisconsin Technical College

An antibiotic crisis is on the rise, only 27 new antibiotics were in clinical development in 2021, which is down from 31 in 2017. This is because the rate at which we are discovering antibiotics is slower than the evolution of microbial antibiotic resistance. Tiny Earth is an organization that relies on the engagement from college students around the world to help discover new antibiotic-producing microbes. It creates a wonderful learning opportunity for all involved in the widespread research. We chose to collect samples of topsoil from a garden in Mosinee, and a subsoil sample from a former bog in New Franken. We chose these areas to involve communities we are familiar with and because we hypothesized due to their characteristics, they would both have antibiotic properties, Mosinee having 12 and New Franken having 2. We collected our soil with sterile equipment, then diluted the soil and plated it using the streak plate method. After inoculating our samples, we tested them against S. epidermidis and Erwinia to observe if our microbes displayed antibiotic activity. At this point neither sample were showing signs of antibiotic material. We each picked one microbe to continue testing with. The next test was against the relatives of ESKAPE pathogens, again this test did not show antibiotic reactions from either sample. It was not until we conducted a PCR and gel electrophoresis that we both received a positive result for the 16S rRNA gene to confirm microbial DNA. Our hypothesis was not correct for the number of antibiotic-producing microbes and some of our research is ongoing.

115 Finding Antimicrobial Properties in Soil Isolates

MADALINE HEIM AND KATELYN NIKOLAI
St. Norbert College

Over the years, there has been an increase in antibiotic resistance. Due to this problem, with the collaboration of the Tiny Earth Project, soil samples from the St. Norbert College campus were collected and tested for antimicrobial properties. This could lead to new therapies for treating antibiotic resistance with positive results from the sample. Bacteria from the soil were isolated from outside of Todd Wehr Hall and cultured in 1/10 LB and 1/10 CYE agar media. Once colonies started to form, the samples were patched to detect possible antimicrobial properties. 13 individual colonies were patched on a lawn for 6 different safe ESKAPE relatives, with 4 colonies showing antimicrobial properties against the safe relatives. PCR was done to amplify 16s rRNA gene that was then sent off for sequencing. Sequencing is being analyzed at the moment and future biochemical testing will better identify the bacteria isolates of interest. moment and future biochemical testing will better identify the bacteria isolates of interest.

116 Exploring Antibiotic Resistant Bacteria

GIANG (GINA) LAI AND TREVOR ONEACRE
St. Norbert College

Naturally occurring antibiotics are becoming more demanded due to the rise in antibiotic resistance among pathogenic bacteria. In alignment with the Tiny Earth Project's goals, bacteria were isolated from the bank of Ashwaubenon Creek's soil and cultured on 1/10 CYE and TS agar media. After isolating 14 colonies, they were patched on a lawn of ESKAPE pathogens to determine possible antibiotic producers. Plate-patching was conducted with 1/10 TS agar media since they were observed to grow better on it. As a result, five colonies were observed to have antimicrobial activities against the pathogen. They were then Gram-stained and examined under the microscope. A PCR was then conducted using two primers: 63F and 1387R. After preparing the 0.7% agarose gel, the PCR products were loaded into the sample wells, and the gel was then run at 80V. As an outcome, one PCR showed a visible band of the expected length. The colony was cleaned and then sent in for sequencing of the 16S rRNA gene we amplified. After obtaining the sequencing results, the gene sequences from both primers were analyzed to identify bacterial species. It was done by comparing the sequences against the NCBI BLAST database.

117 Anthropogenic Activity Lowers Rates of Antibiotic Production in Soil Bacteria

KIERA ORTIZ, HOLLY AHLSWEDE, OLIVIA NEUBAUER, VERONICA MCHENRY
University of Wisconsin - Madison

The increase in antibiotic-resistant infections is a growing threat to human health and pharmaceutical companies have failed to reinvest money into the development of new antibiotics. Samples of bacterial colonies from soil with high human activity and low human activity were plated against ESKAPE strains to analyze the number of antibiotic-producing colonies. Bacterial colonies sampled from soil with low levels of human activity were shown to have a higher rate of antibiotic production than bacterial colonies sampled from soil with higher levels of human activity (Fisher Exact Test $p=0.006$). We speculate on potential explanations for this result, including soil health, microbial diversity, and the lower metabolic activity of microbiomes with increased levels of human activity.

STUDENT POSTERS

118 Aiding Antibiotic Resistance Through Bovine Soil Sampling

DOMINIQUE PAVLAT
Northeast Wisconsin Technical College

When antibiotics were first discovered, their limitations were immediately known. In 1928, Alexander Fleming discovered the first antibiotic, penicillin. Antibiotics were misused and over prescribed for decades, leading to the modern-day prevalence of antibiotic resistance seen in multiple bacterial species. To assist in solving this problem, this study focuses on obtaining a soil sample and testing bacteria present in the sample for novel antibiotic properties. The soil sample was obtained six inches below ground in a cow pasture, where high microbial concentrations are expected. The sample was then diluted and inoculated onto media to test if antibiotic properties were present against other strains of bacteria. Bacterial colonies that displayed these properties were then tested against ESKAPE pathogens, prevalent antibiotic-resistant bacteria that pose a risk to public health, as determined by the World Health Organization, WHO. Results identified three different colonies that displayed potential antibiotic properties. The colony that displayed the most prominent zone of inhibition was selected and further tested for a novel antibiotic.

119 Finding Antimicrobial Properties in Soil Samples of Green Bay

HAILEY JOHNSON, MACY EDWARDS
St. Norbert College

As antibiotic resistance is becoming a more precarious issue, St. Norbert College's BIOL350 students have paired up with Tiny Earth Project in hopes to broaden the collection of antimicrobial producing bacteria. Bacteria were isolated from soil collected from Fonferek's Glen Brown County Park in Green Bay, Wisconsin, and isolated on 1/10 TS agar media. Bacteria were then patched onto an ESKAPE pathogen safe relative, Acinetobacter baylyi. One colony showed antimicrobial properties and appeared translucent and colorless, with a round, umbonate shape, smooth margins, and a smooth, glistening surface. Bacteria that showed such properties were then isolated, ran through PCR, and sequenced (16s rRNA) by MC Lab.

120 Tiny Earth at St. Norbert College: Potential Antibiotic Producers in Plant Soil

HAILEY JOHNSON, MACY EDWARDS
St. Norbert College

In today's world, we have to be smarter than the rapidly-evolving illnesses that infect the human population. Some microorganisms produce natural antibiotics, and these can be useful for combating some of these illnesses. Using Tiny Earth protocol, bacteria were isolated from houseplant soil. Once there was some growth from the bacteria in the soil, 26 total different colonies were patched on plates of 1/10 TS (14) and LB (12) agar media and then tested against ESKAPE safe relatives. Two colonies exhibited potential inhibition of the pathogens. One of the colonies is gram-negative rods that have a clear appearance. The other is also a gram-negative rod but the colonies are orange in appearance. These two potential antibiotic-producing colonies were put through a series of tests. The first test was to perform colony PCR to amplify the 63F and 1387R genes in the bacteria. Then, gel electrophoresis was run to determine molecular weight of the DNA in the bacterial samples. Finally, PCR cleanup was performed in order to allow for successful sequencing. A 16S sequence was obtained from MCLab. Next, the results of the tests will be used to determine the identity of the isolated bacterial cultures.

121 The Impact of Vitamin C on Bacterial Growth and Production of Secondary Metabolites with Antibiotic Properties

MARCI FEIDT, CAELEY ANDERSON, ABIGAIL KOHLER AND LILY TARRANT
St. Norbert College

Tiny Earth is a research lab dedicated to antibiotic discovery in an attempt to combat the ongoing global antibiotic crisis. This experiment in particular aimed to determine how varying concentrations of vitamin C can influence the yield of antibiotic-producing secondary metabolites, observed through zones of inhibition from bacteria extracted from soil. We found no statistical significance between varying vitamin C concentrations and the number of zones of inhibition. Although no statistically significant relationship was found between the concentrations of vitamin C and the production of antibiotic-producing secondary metabolites, we still were able to identify 40 antibiotic-producing colonies, and a subset were selected for further evaluation.

122 Discovery of Antibiotic-Producing Bacteria: Bacillus toyonensis and Bacillus paramobilis

HUNTER MOORE
Concordia University Wisconsin

As a result of poor antibiotic stewardship, bacterial resistance to such medications has become a global problem impacting a major avenue of treatment for hospitals. To combat this healthcare crisis, the students of Concordia University Wisconsin have been in search of new antibiotics isolated from bacteria extracted from soil. The location of the collected samples ranged from wooded nature trails to swamplands. Given the moist environment of these locations, it was hypothesized that the ideal growth conditions would foster a habitat rich for antibiotic producing bacteria. The gathered bacterial samples were grown and isolated on Tryptic Soy Agar before being tested against other known pathogenic microbes. The potentially antibiotic-producing samples were then sequenced via a 16s PCR. The bacteria identified were *Bacillus toyonensis* and *Bacillus paramobilis*, which have not been previously tested for antibiotic production. been in search of new antibiotics isolated from bacteria extracted from soil. The location of the collected samples ranged from wooded nature trails to swamplands. Given the moist environment of these locations, it was hypothesized that the ideal growth conditions would foster a habitat rich for antibiotic producing bacteria. The gathered bacterial samples were grown and isolated on Tryptic Soy Agar before being tested against other known pathogenic microbes. The potentially

antibiotic-producing samples were then sequenced via a 16s PCR. The bacteria identified were *Bacillus toyonensis* and *Bacillus paramobilis*, which have not been previously tested for antibiotic production. These two samples were further tested for their secondary metabolites and other cellular functions.

123 From Soil to Solution: The Potential of Marshland Bacteria Against Antibiotic Resistance

PAIGE WEBER
Concordia University Wisconsin

This study was conducted in response to the increasing prevalence of antibiotic-resistant bacteria. We aimed to identify potential new antibiotics by analyzing soil bacteria for antibacterial production. In this study, we isolated bacteria from soil samples collected from marshland on the Concordia University Wisconsin campus and cultivated the bacteria on Tryptic Soy Agar (TSA), to potentially identify antibiotic-producing bacteria. The marshland was selected due to its ability to host a diverse microbial ecosystem, which we hypothesized would foster competition resulting in antibiotic-producing bacteria. After inoculating the TSA plates with the environmental samples, the growth of multiple bacterial colonies was observed. These colonies were then isolated and tested against human bacterial pathogens: *S. aureus, B. subtilis*, *E. coli*, and *S. typhi*. Three of the bacterial colonies had zones of inhibition indicative of antibiotic production. This discovery highlights how environmental samples can be valuable resources for finding unique antibiotic-producing bacteria, which could contribute to the development of new antibiotics.

124 Antibiotic Producing Microorganisms in Soil with Higher Water Levels

ALEXANDRIA EHLERT
College of Menominee Nation

As we all know, climate change is rapidly altering the planet as we know it. Whether that be rising water levels or heightened bacterial growth, this study sought to identify how water levels in soil affect the bacterial landscape. Soil samples were collected from an area where native plants are growing in Northeast Wisconsin near the Wolf River. Unfortunately, we had a particularly dry fall and had to replicate a rain event in the lab using rainwater collected separate from the soil. Samples were grown and tested on tryptic soy agar using Tiny Earth protocols. All samples were tested against *E. coli* and *E. aerogenes*. This study found no potential antibiotic producing bacteria, however, further testing of samples will need to be tested against other safe ESKAPE pathogens to confirm antibiotic production is present. This study may advance further climate research and grant funded projects at the College of Menominee Nation.

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SYMPOSIUM ORGANIZERS

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Associate Professor and Chair of Human Biology
UW-Green Bay

SAMANTHA BETANCUR
Camps and Outreach Coordinator
CSET, UW-Green Bay

LUCY FENZL
Biology Science Faculty
Dean of Academic Success
College of Menominee Nation

LAURA LANGLEY
Project Manager
Tiny Earth, UW-Madison

DAVID HUNNICUTT
Professor of Biology
St. Norbert College

ELIZABETH PAUL
Annual Giving Manager
UW-Green Bay

ANGELO KOLOKITHAS
Professor of Microbiology
Northeast Wisconsin Technical College

KENLYN PETERS
Biology Faculty
Northeast Wisconsin Technical College

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Director, Tiny Earth
Tiny Earth, UW-Madison

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