tiny earth

12|09|2022

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 14,000 students per year who are contributing to studentsource new antibiotic discovery from soil. Tiny Earth is expanding rapidly throughout Wisconsin. To date, 26 Wisconsin colleges, universities, and high schools have partnered with Tiny Earth to identify new life-saving antibiotics produced by soil bacteria, which have historically proven to be the most productive source of new antibiotics.

SCHEDULE OF EVENTS

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

5 p.m. » Symposium Opens/Welcome

» **H. Jeffrey Rafn**
President, Northeast Wisconsin Technical College

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

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

» Land Acknowledgement, **Catishe Grignon**

» History of Tiny Earth

» **Dr. David Andes**
KEYNOTE: Drugs from Bugs of Bugs -
New Antibiotics to Fight the Resistance Pandemic
William A. Craig Professor
Department of Medicine
Department of Medical Microbiology and Immunology
Chief, Division of Infectious Diseases
Director, Wisconsin Antimicrobial Discovery and Development Center
School of Medicine and Public Health and School of Pharmacy
University of Wisconsin-Madison

6:15 p.m. » Student Poster Presentation

7:20 p.m. » Closing Remarks

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SPEAKERS

H. Jeffrey Rafn

President, Northeast Wisconsin Technical College

Dr. Jeff Rafn has been president of Northeast Wisconsin Technical College (NWTC) since 1997. Under his leadership, the 100-year-old college has been transformed into a team-based, learner-centered organization offering industry-leading new facilities in health sciences, energy, IT, public safety, advanced manufacturing and more. During his tenure, the College has been nationally recognized for innovative student success solutions by the Lumina Foundation, Achieving the Dream, Gates Foundation, National Science Foundation, Bellwether Consortium and American Association of Community Colleges (AACC). Rafn himself received the 2015 Pacesetter of the Year award from the National Council for Marketing and Public Relations (NCMPR) and the 2016 Giving Back Award for Presidents and Chancellors from INSIGHT into Diversity magazine. He received the VISTAGE 2019 Lifetime Achievement Award and the 2019 WTCS Eagle Management Award. Rafn serves on the executive board of Community Colleges for International Development and has served multiple terms on the Workforce Development Commission of the AACC. He is a champion of industry and K-12 partnerships, and he has been instrumental in the development of the NEW Manufacturing Alliance, which has over 200 members. Dr. Rafn came to NWTC in 1997 from New Hampshire, where he had been state commissioner of the New Hampshire Community Technical College System. He has a doctorate in sociology from Boston University.

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.

tiny earth

studentsourcing antibiotic discovery

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.

Catishe Grignon

Student, College of Menominee Nation

My name is Catishe Grignon, I am Native American and I grew up on the Menominee Indian Reservation. I am currently a junior at the College of Menominee Nation and my major is in Biological and Physical Sciences. I love to participate in CMN's extra curricular activities and I also love to travel!

Dr. David Andes

William A Craig Professor in the Departments of Medicine, Medical Microbiology and Immunology, Head of the Division of Infectious Diseases at the University of Wisconsin, and Director of the Wisconsin Antimicrobial Drug Discovery and Development NIH Center of Excellence.

The focus of Dr. Andes' research program strives to identify strategies to combat antimicrobial drug resistance. His study tactics span from the bench to the clinic, including identifying biofilm resistance mechanisms, drug discovery and development, delineating the optimal dosing strategies for treatment of drug resistant infections using pharmacometric approaches, and clinical trial study of epidemiology and therapy of drug resistance.

STUDENT POSTERS

1 Variations in Microbial Growth Between Early Fall and Late Fall Sampling

Catishe Grignon
College of Menominee Nation

There are many medicinal plants located throughout the Menominee reservation. Using two different plants, Plantain and Mullein, located on the College of Menominee Nation Phenology trail, soil samples were collected from each plant site, then tested. Soil collected during late October from these two phenology trail plants were tested on two different growth media; tryptic soy agar and nutrient agar. Neither media was preferred, at the same time using Tiny Earth protocols. This study found no potential antibiotic producing bacteria at either plant site, while samples from previous research conducted in August produced several antibiotic producing bacteria. This study may advance further climate research and grant funded projects at the College of Menominee Nation. This study will also help reinforce the medicinal uses of these plants.

2 Researching Bacteria in Soil

Sierra LeRoy
Green Bay West High School

Tiny Earth is a global network of students and instructors conducting research on soil and antibiotics. We started off with collecting soil samples on the UW-Green Bay campus, (44.5290329,-87.9155494). One gram of soil was serially diluted in water on Luria Broth (LB) agar plates containing cycloheximide. Isolates were picked and patched to create master plates. We then screened each isolate for antibiotic production. For this lab, we tested master plate colonies for antibiotic production against *Staphylococcus epidermidis* and *Escherichia coli (E. coli)* on different LB agar and potato dextrose agar (PDA). Two isolates showed antibiotic activity against *B. subtilis* on PDA. Tiny Earth educates students the importance of soil and antibiotics, where students get the opportunity to do hands-on labs, while learning the importance of the topic.

3 Tiny Earth Big Soil

Claire Pasterski
Green Bay West High School

Tiny Earth is making a big impact, by giving high school students the opportunity to learn about soil bacteria that produce antibiotics to treat resistant infections. A soil sample located at the foot of a tree on the UW-Green Bay campus was collected (44.5303350, -87.9203720). The sample was serially diluted in water and plated on Luria Broth (LB) agar plates generating 7.5x10-3 CFU/g. Isolates were picked and patched to LB agar and Potato Dextrose Agar (PDA) to create master plates. Isolates were patched to LB agar and PDA plates containing either *Escherichia coli (E.coli)*, *Bacillus subtilis* (*B. subtilis*), or *Staphylococcus epidermidis* (*S. epidermidis*). One isolate showed antibiotic production against *S. epidermidis* on PDA .

4 Combating Resistant Bacteria

Olivia Vang
Green Bay West High School

Modern antibiotics can no longer combat many infections caused by resistant bacteria. We collected soil on the campus of UW-Green Bay (44.53*N, -87.92*W). The sample was serially diluted in water and plated on Luria Broth (LB) agar plates generating 6.0 x 10*5 CFU/g of bacteria. Isolates were transferred to LB agar and PDA to create master plates. To test for antibiotic activity, isolates were patched to LB agar and PDA agar plates containing either *S. epidermidis, B. subtilis* or *E. coli*. One isolate showed antibiotic activity against *E. coli*.

5 Saving the World One Antibiotic at a Time

Asia Chevez
Green Bay West High School

Tiny Earth is a global network of students and instructors conducting research on antibiotic-producing soil bacteria. A soil sample was collected from the UW-Green Bay campus (44.52435° N,87.92115° W.) One gram of soil was diluted in water and plated on Luria Broth (LB) agar plates containing cycloheximide yielding 4.0*10^10 CFU/g. Next, isolated bacteria were picked and patched to create master plates. Soil isolates were then patched to Potato Dextrose Agar (PDA) and LB agar plates containing either *Escherichia coli (E. coli)* or *Bacillus subtilis* (*B. subtilis*) to test for antibiotic production. One isolate displayed antibiotic activity against *E. coli* on LB agar. Future tests will be completed to determine the genus of the bacterium.

STUDENT POSTERS

6 Antibiotic-Producing Soil Bacteria

Fatuma Umberwa
Green Bay West High School

Tiny Earth research identifies and studies antibiotics produced by soil bacteria to address the world crisis of antibiotic resistance. A soil sample was collected on the UW-Green Bay campus (44.52991° N -87.92192° W). One gram of soil was diluted in water and plated on Luria Broth (LB) agar plates to isolate bacteria. To assess antbiotic actvity, soil bacteria were patched to potato dextrose (PDA) and Luria Broth (LB) agar plates containing either *E. coli*, *B. subtilis*, or *S. epidermidis*. Six out of 9 isolates showed antibiotic activity. Lastly, the 16S rRNA gene was amplified in each isolate using a polymerase chain reaction to identify the genus of our antibiotic producers.

7 A "Groundbreaking" Discovery of Antibiotic actvity

Maryah Smith
Green Bay West High School

Tiny Earth's main purpose is to find and identify antibiotics to address the world crisis of antibiotic resistance. A loamy soil sample was collected on the UW-Green Bay campus located at GPS coordinates, (44.52967 N, 87.91758 W.) The soil sample was serially diluted in water and plated on Luria Broth (LB) agar plates. The colony forming units per gram (CFU/g) was calculated to be (100,000,000 CFU/g.) The isolates were picked and patched to create master plates for additional tests. Then, the isolates were patched to LB agar plates and Potato Dextrose Agar (PDA) plates containing either *Escherichia coli (E. coli)* or *Bacillus subtilis* (*B. subtilis*.) One isolate inhibited growth of *E.coli* on PDA. Future tests will be done to determine the genus of the bacterium.

8 Soil: Medicine's Powerful Ally

Stacy Martinez
Green Bay West High School

The purpose of the Tiny Earth initiative is to create an opportunity for students to contribute to the research of microbiology by looking for antibiotics in soil samples. The soil sample was taken from the campus of the University of Wisconsin-Green Bay, GPS coordinates (-44.5304287, -87.9195493.) One gram of soil was serially diluted in water to isolate bacteria on Luria Broth (LB) agar aplates. Colony forming units per gram (CFU/g) were calculated to be 4,000,000 CFU/g. Isolates were patched to Potato Dextrose Agar (PDA) plates containing test strains of bacteria to evaluate antibiotic actvity. One isolate displayed antibiotic actvity against *Staphylococcus epidermidis (S. epidermidis.)* Future tests will be conducted to determine the genus of the bacterium.

9 The Future of Antibiotics

Sophie Hoffman
Green Bay West High School

Tiny Earth is a program to inspire young scientists to learn more about antibiotic-resistant bacteria and antibiotic discovery. The goal of this research is to find new antibiotics that will be effective against resistant strains. Soil samples were collected from the UW-Green Bay campus (44.52931*N, 87.91782*W.) The colony forming units per gram (CFU/g) was calculated to be 3x10^10 CFU/g. The isolates were then picked and patched to create master plates. The isolates were then patched to Luria broth agar (LB) and Potato Dextrose Agar (PDA) plates containing either *Escherichia coli (E. coli)* or *Bacillus subtilis* (*B. subtilis*.) Three isolates inhibited the growth of *B. subtilis* on the LB agar. Future tests will be done to determine the genus of the bacteria.

10 A solution underneath your feet

Suabnag Kong
Green Bay West High School

Tiny Earth is a research program for student sourcing antibiotics to solve the world crisis of antibiotic resistance. Soil was collected on the UW-Green Bay campus (44.52806 N, 87.91857 W), diluted in water and plated on Luria Broth (LB) agar containing cycloheximide, yielding 6 x 1010 CFU/g. Individual soil isolates were patched to potato dextrose agar and LB agar plates containing either *Escherichia coli (E. coli)*, *Staphylococcus epidermidis* (*S. epidermidis*) or *Bacillus subtilis* (*B. subtilis*) as test strains to evaluate antibiotic activity. Results will be shared at the symposium.

11 Antibiotic Producers Collected from Richland Center & Green Bay

Courtney Malnory, Olivia Duell
Northeast Wisconsin Technical College

Scientists for many years have faced problems with antibiotic-resistant pathogens and finding antibiotics to fight certain diseases. These problems have led to consequences of mortality around the world. The Tiny Earth organization inspires students to help with the issue of finding effective antibiotics due to the current decreasing supply. This project inspires students to do their research on soil bacteria to discover antibiotics in their environment and community. For our project, we used two soil samples from different areas. One sample was taken from the base of a tree two minutes away from NWTC (Northeast Wisconsin Technical College) in the backyard of the student's apartment. The other sample was taken from the other student's tree in her front yard in her hometown of Richland Center. The purpose of selecting from these locations was to compare soil samples from somewhere more populated versus somewhere less populated, essentially country vs city areas. We predicted that the less populated area would produce more antibiotics because of how untouched it was and how much mold was present on the tree where the sample was collected. The methods we used involved collecting soil and bringing it to class. Once in class, we did a series of tests to isolate our soil samples and to determine if we had any antibiotic producing bacteria. Our experiment showed that while both soil samples had antibiotic-producing bacteria, the more populated (the city) sample had more antibiotic-producing bacteria. This means our hypothesis was incorrect. Further experiments are being run to determine more details about the antibiotic producing bacteria, so some results are to be determined.

12 Antibiotic Producers Collected from Aurora Bay Care Medical Clinic and St. Mary's Hospital Soil

Brenna Schlader, Asia Schwantes
Northeast Wisconsin Technical College

The transfer of resistant bacteria to humans by farm animals was first noted more than four decades ago. Since then, scientists have dealt with the problems caused by these antibiotic-resistant bacteria while also trying to find new antibiotics to keep up. Antibiotic resistance is a worldwide health problem that is only getting worse over time Thankfully, the Tiny Earth Organization granted students the opportunity to examine soil samples within our local communities to possibly discover new antibiotic-producing microbes. We chose to do scientific research on two hospitals' soil: Aurora Bay Care Medical Center and St, Mary's Hospital. We hypothesized that Aurora Bay Care Medical Center would have more antibiotic-producing properties compared to St. Mary's Hospital since Aurora Bay Care Medical Clinic's soil seemed to fit the properties necessary for microbial growth. Aurora Bay Care Medical Clinic's soil was located in a more aquatic environment, therefore, holding the moisture that is a critical factor toward microbial multiplication and growth. To begin our research, we diluted both soil samples and plated our dilutions using the streak plate method. The microorganisms found were further inoculated with specific agar and screened against *S. epidermidis* and *E. carotovora* to illustrate which microbes were emitting antibiotic-producing activity. We discovered that St. Mary's Hospital only possessed one possible antibiotic producer while Aurora Bay Care Medical Center possessed two. However, when both sites' antibiotic-producing microbes were tested against ESKAPE pathogens, the antibiotic-producing microbe from St. Mary's hospital produced such properties toward both *S. epidermis* and *E. carotovora*. Aurora Bay Care Medical Clinic displayed properties toward only *S. epidermidis*. Our hypothesis was incorrect, as Aurora Bay Care Medical Clinic produced fewer antibiotic-producing properties when compared to St. Mary's. Even though Aurora Bay Care Medical Clinic possessed two possible antibiotic-producing microbes, it only showed activity toward one bacterium. Our research of St. Mary's Hospital and Aurora Bay Care Medical Clinic antibiotic-producing soil is still ongoing; therefore, some results are to be determined.

13 Antibiotic Producing Microbe Collected from Beach soil by the YMCA in Manitowoc

Natasha Flores
Northeast Wisconsin Technical College

There hasn't been a new antibiotic discovered for the past few decades. People are getting sicker faster and dying earlier due to the antibiotic resistance that has developed due to overuse of current antibiotics. If there are few antibiotics that work and inability to find new ones the death rate will continue to grow catastrophically around the world. The purpose of this project is to potentially find a new antibiotic producing microbe in the community. My hypothesis is that the beach by the YMCA in Manitowoc would have an antibiotic producing microbe due to the environment of sandy wet soil close to the Lake Michigan water. The sample of soil was diluted to isolate microbes and grown using the streak plate technique. This sample was then inoculated and screened against *S. epidermidis* and *Erwinia* to see which microbes had antibiotic producing activity against these bacteria. I ended up having one microbe that was a double producer, so naturally I decided to stick with this microbe to do further testing. This microbe was then screened against the ESKAPE pathogens, of which there was antibiotic clearing activity on 7 of the 8 ESKAPE pathogens. This microbe is looking promising and is now being used for further testing, therefor some results are to be determined.

14 The Wonderful Parks Around Our City

Gabbie Wares, Jenna Margetta, Lillian Skaggs
Northeast Wisconsin Technical College

When an individual gets sick from a bacterial infection, antibiotics have undoubtedly changed the lives of many people by saving them from early death. Antibiotic resistance refers to the ability of bacteria to alter their genetic material and mutate to avoid destruction by antibiotic medications. The Tiny Earth organization has given the students a great opportunity to research soil from places within their own community to find new microbes and present the findings at the conference this year.

Our research focuses on soil samples taken from different parks. One park was in Marinette, Wisconsin called Red Arrow Park, while the other two were from Menominee called Henes Park and Escanaba, Michigan called Ludington Park. The type of environments we gathered the soil from varied. The sample that came from the park in Menominee was from a forested wetland. Compared to the other samples, this soil sample had the most water content which gave the microbes a good chance to grow and multiply. Our hypothesis was this sample would produce the most antibiotics against the bacteria.

In the lab, we took the samples and diluted them, then we spread the samples of different dilutions all over the plates. Twenty unique bacteria were selected to be tested against *Erwinia* and *Staphylococcus*. Henes Park results were no double producers and only had one antibiotic activity on the *Staphylococcus* plate. Ludington Park results were no double producers and antibiotic activity on one of the *Erwinia* plates. After viewing our plates after the antibiotic screening lab, the group noticed that the wetland soil had five double producers from the *S. epidermidis* and *Erwinia* plates.

This means our hypothesis was correct. Our research is still ongoing; therefore, some results are to be determined.

15 Do You Know What Is in Your Dirt?

Mary Yang, Emma Davis, Caroline Basten, Samady, Romo Cortes, Mary Yang
Northeast Wisconsin Technical College

There is a medical crisis with bacteria resistant to antibiotics. Antibiotic resistance is the observation that bacteria reduce the effectiveness of the drug that was designed to help destroy bacteria. Human misuse has been determined to be the driving force of antibiotic resistance. Our mission is to discover bacteria that produce antibiotics in the soil. The following locations were chosen to find antibiotic-producing bacteria: a cornfield, deer hunting spot, cabin, and Bay Beach. Our group's hypothesis was that Bay Beach would have the most antibiotics out of the four locations. The reason for our prediction was because Bay Beach has a lot of traffic with humans and animals which would have more antibiotics around. In addition, the soil at Bay Beach contained moisture because the soil was collected by water. We collected soil samples from our locations and performed soil dilution tests to isolate single colonies. Twenty colonies were selected for testing against *Erwinia carotovora* and *Staphylococcus epidermidis*. The results indicated that samples from Bay Beach and the corn field had only one antibiotic producing bacteria each. The deer field sample had three and the plate with the most antibiotic activity was the cabin sample with antibiotic producers. Upon testing with other ESKAPE pathogens, only the cabin and cornfield had additional production activity. Our group agreed that our hypothesis was incorrect. We thought that Bay Beach sample would have the most activity with having a lot of traffic with people and animals, but our results showed that the cabin sample had the most antibiotic activity. The cabin showed additional activities against the ESKAPE pathogens *E. coli*, and *B. subtilis*. Other experiments are ongoing to determine if the bacteria isolated from these samples are known to produce antibiotics.

16 Just Goating Around

Jamie McLaughlin, Jaimie Yunk
Northeast Wisconsin Technical College

For the past few decades, scientists have been faced with the problems of antibiotic-resistant pathogens and the inability to find novel antibiotics. These problems have led to an increase in mortality around the world. The Tiny Earth organization presented the opportunity to research soil in our communities to find new microbes that release antibiotics and present our findings at a conference. We have decided to evaluate two diverse types of hobby farms to see if there is antibiotic production in the soil to justify that there is a beneficial microbe in the hobbies people find pleasure in. One soil sample came from a farm where goats had less area to roam, whereas the other sample came from a more undeveloped area to roam. We had anticipated that the soil sample from the more free-range goats would have more likelihood of antibiotic-producing microbes due to having more access to organic consumption such as grass. Since the COVID pandemic, there has been a rise in hobby farms. If antibiotics are present in the soil, it would allow there to be many different areas to get samples from. We were able to study the soil samples by isolating the bacteria. We are still working on the soil sample currently, but the experiment did show that the goats with only access to store-bought food did have more antibiotic producing microbes in the soil. This means our hypothesis was not as we anticipated. More experiments are being run to determine the exact nature of the bacteria in the soil samples, so the results will be available at a later date.

17 Exploring Antibiotic Producing Microbes from Local Elementary Schools

Jordyn Pegel, Isabella Wiezbiskie
Northeast Wisconsin Technical College

Did you know more than 2.8 million antibiotic-resistance infections occur in the United States each year, with more than 35,000 deaths due to this phenomenon, according to the CDC. 35,000 is twice the seating capacity of Fiserv Forum in Milwaukee, WI. So that is why NWTC has partnered with the Tiny Earth organization to help fight the health crisis of our decreasing supply of effective antibiotics from misuse. Our class has divided into groups to research antibiotic-producing bacteria by taking a deeper dive into the bacteria living within the soil. Our partnership chose to retrieve soil from local school playgrounds due to the amount of child activity. Many surveys stated that children do not follow proper hand-washing techniques and hygiene in school. Therefore, germs and bacteria rapidly travel around elementary schools and get distributed throughout the children and equipment during the school day when they engage in daily activities such as playing on the playground. We suspect Webster Elementary will have the most antibiotic-producing microbes because the playground activity volume is higher than Richmond Elementary. We first collected our samples from each location to bring to class. Once the samples were in class, we diluted our soil samples. Next, we selected 20 separate colonies to test against *S. epidermidis* on and *Erwinia*. Richmond elementary had antibiotic producers against *Erwinia* and Webster elementary had 4 antibiotic producers on *S. epidermidis* and 3 on the *Erwinia* plate. Webster Elementary had two double producers within the mix. After those results, we continued to test our antibiotic producing microbes on the ESKAPE pathogens. Richmond elementary had positive results for *S. epidermidis* and *E. aerogenes*. Meanwhile Webster Elementary had positive results for *S. epidermidis* and *A. baylyi.* Lastly, we started genotypic testing, and those results are still pending.

18 Tiny Earth: A Dirt Discovery

Kali Cox
Northeast Wisconsin Technical College

Antibiotic resistance, two words that, separately, general society does not give much thought to; but when combined in the same sentence, they become the phrase that plagues the 21st century and threatens the very medical community and it's ability to treat even the simplest of infections. The Tiny Earth Project allowed the opportunity to use different testing methods to explore soil from my place of choice, outside of the fence of Austin Straubel Airport. My hypothesis was that I would be able to find at least one antibiotic producing microbe. I developed this hypothesis due to the amount of traffic that the airport sees daily from different types of travel and the chemicals and procedures used throughout the year, but especially during snow. Through several dilution and plating steps, my bacteria showed some antibiotic producing capabilities against both *S. epidermidis* and *Erwinia*. Additionally, after more inoculation steps against pathogens with no known antibiotic cure, also known as the ESKAPE pathogens, my sample showed those same antibiotic capabilities against two of the six pathogens. At this time, it appears that my hypothesis was correct. My research is still ongoing at this time, therefore some results are still to be determined.

STUDENT POSTERS

19 Wisconsin's Soil and its Contribution to Antibiotic Research World-Wide (From the Perspective of 3 NWTC Students)

Jose Ibarra, SamanthaYang, Haley Miller
Northeast Wisconsin Technical College

In the last few decades, humankind has been unable to find novel antibiotics and this has become a major barrier to achieving further progress in the medical world. If we are unable to find new antibiotics soon, we might repeat history and see infections arise that we are no longer able to treat. The Tiny Earth Project has allowed us, and millions of students worldwide aid in the research of new organisms that can help us get back on track.

Our group collected soil from sites that are relevant to us. From farm soil, to the highly organic shores of Lake Michigan, and finally the soil adjacent to a nursing home, our hypothesis was that such locations could be suitable for the kind of organisms we were looking for, as the organic contents found in these sites, along with the presence of pre-manufactured drugs from the nursing home soil, would result in all of us obtaining organisms with antibiotic-producing capabilities.

We then diluted our samples to different concentrations (1/1,000 to 1/1,000,000) and, through the streak-plate method, inoculated our samples on agar-based plates. We let the organisms grow and then we adjusted our culture conditions to see if we could get different results. These resulting samples were screened against *S. epidermidis* and *Erwinia*. We then tested them against other relatives to the ESKAPE pathogens. Our collective results showed that we had four antibiotic producing microbes, with one double-producer. Our hypothesis was correct as we were able to find organisms that adapted well in all areas we tested; however, our research is still ongoing.

20 Searching for antibiotic producing bacteria

Alison Cox
Northeast Wisconsin Technical College

Antibiotic misuse has created many bacterial pathogens that are resistant to current medications. The biggest threats are called the ESKAPE pathogens. Options to treat resistant infections are decreasing since pharmaceutical companies have stopped research. The purpose of the Tiny Earth project is to find antibiotic activity in bacteria found in soil with the hope that it may lead to new antibiotics to combat ESKAPE pathogens. I hypothesized that I would have at least one organism. I collected soil using a sterile scoop, then the sample was diluted and placed on media to grow. Twenty colonies were screened against safe alternatives to the ESKAPE pathogens. I had one colony that showed promise when screened with *E. carotovora* and again when screened with *B. subtilis*. My hypothesis was correct and I did have one. Tiny Earth project really could lead to a new discovery that will defeat the ESKAPE pathogens.

21 Soil Antibiotics Collected from Fox River Mall & Bay Park Square Mall

Brianna Backhaus, Roxanna Lopez
Northeast Wisconsin Technical College

According to the Center for Disease Control and Prevention, 1.27 million people died in 2019 due to having a microbial disease that was resistant to antibiotics. We may not like to admit it but the world we live in is surrounded by microbes. Antibiotic resistance has become an increasing dilemma that needs to be addressed immediately. To combat this ever-growing problem, the Tiny Earth organization provided an opportunity to college students around the world to explore and examine the soils in their communities to discover virgin microbes that have yet to be determined to produce antibiotics. To spark people's interest, our spots of scrutiny were the Bay Park Square mall in Green Bay and the Fox River Mall in Appleton. The hypothesis we constructed was that the Bay Park Square Mall will contain the most antibiotic producing bacteria because there is less foot traffic, meaning less people visiting and disturbing the soil. It is also closer to Lake Michigan, so the microbes have a more satisfactory environment to grow in. For these labs we used a multitude of techniques to isolate the microbes. Starting off we diluted our samples to plate them while using the streak plate method. Next, we studied what grew, inoculated and screened the growth against the *S. epidermidis* and *Erwinia* to see which samples produced antibiotics. The Bay Park Square Mall only had one antibiotic producer while the Fox River Mall had two and both were dual producers. Then we chose one of the antibiotic producers to screen against the ESKAPES pathogens. We found that the Fox River Mall sample produced antibiotics against six ESKAPE pathogens while the Bay Park Square mall sample had activity against two ESKAPE pathogens. Our hypothesis was incorrect because the Fox River Mall ended up generating more antibiotic producing microbes. Research of the two sites is still underway and will be shared in the future.

STUDENT POSTERS

22 Analysis of Soil from Farmland and Old Mining Ground

Summer Bourg
Northeast Wisconsin Technical College

For decades scientists have been faced with the issue of antibiotic resistance and the ability to find new antibiotics. Problems such as these have had and will continue to have devastating consequences, such as increasing mortality rates from bacterial infections. Tiny Earth is giving college students the chance to collect and test soils for microbes that release antibiotics in hopes to help the antibiotic resistance problem. To aim for a diverse sample, I chose to take soil from farmland which is also located around old iron ore mining land. My hypothesis was that the soil would contain four antibiotic producing microbes. After collecting the soil, I diluted and plated the sample using the streak plate technique. The microbes were then inoculated and screened against the bacteria *S. epidermidis* and *Erwinia* to determine if they had any resistance against the bacteria. The results showed that one microbe had antibiotic activity against *S. epidermidis*. I then screened the microbe against relatives of the ESKAPE pathogens. The results showed that my soil sample contained one antibiotic producing microbe. My hypothesis was wrong, my soil sample only contained one antibiotic producing microbe and not four. More will be determined as my research is still ongoing.

23 Soil From Farmland and Its Antibiotic Potential

Makayla Kesler
Northeast Wisconsin Technical College

Due to the increase in antibiotic resistance, there are now not enough antibiotics to work against all the different types of bacteria. Scientists are completing research to discover new antibiotics and help with this issue. NWTC's Microbiology students completed collection and experiments to find soil samples that have bacteria with antibiotic properties. I collected a specimen from my horse pasture and predicted that it would have two different colonies with antibiotic properties. First, I collected a dirt sample with a clean scoopula and made sure not to contaminate the sample. After diluting the sample, it was spread on a media plate to promote bacteria growth. Changes were made to grow select colonies and the growth process was repeated multiple times. My sample grew colonies that showed activity against *Erwinia* bacteria spread on them, but when tested against multiple other bacteria samples the antibacterial properties were unable to thrive. Although it did create a zone of inhibition for one bacterium, it showed no antibiotic activity. This means my hypothesis was incorrect and my sample did not have two strong colonies with antibacterial properties. My experiment is ongoing.

24 Antibiotic Producer Discovery from Beyond the Grave

Ashton Faulkender
Northeast Wisconsin Technical College

Antibiotics are an integral aspect of modern medicine, and without them our current life expectancy would be much lower. Unfortunately, the killer microbes these antibiotics fight against are ever evolving and eventually become resistant to antibiotics of years prior. Thus, we must find new antibiotic producing bacteria and fortunately anyone can do this, even in their own backyard soil. In this experiment I am attempting to find, isolate, and identify new potential antibiotic producing microbes. I chose to collect soil near a cemetery, as it would make sense that antibiotic producing microbes would live in an environment of decomposition. I predicted that I would discover five new antibiotic producing microbes from my selected soil location. I collected my soil and diluted it enough to isolate twenty unique colonies of microbes. Then I screened these twenty unique microbes against *S. epidermidis* and *Erwinia*. I found that I had only two antibiotic producing microbes in my culture. I had only found two, but one of those was a dual producer. I chose this microbe from my master plate to continue my testing on ESKAPE pathogens. My hypothesis was not correct, though I had still managed to find a promising antibiotic producer to continue my research. Our next step is to identify our microbes genetically, in hopes that we can discover something brand new for the betterment of mankind.

25 Antibiotic Producers from Voyageur Park & Bruemmerville Park Soil

Cyana Soung
Northeast Wisconsin Technical College

The discovery and importance of antibiotic use to treat infection have saved countless lives. Over time, some bacteria develop resistance to antibiotics. Resistance occurs through mutation or gene transfer, due to the overuse and misuse of antibiotics. These antibiotic-producing bacteria are what we're researching in The Tiny Earth project. The Tiny Earth organization allows students the chance to research antimicrobial production in soil samples directly from our communities. For our research, two soil samples were collected from Bruemmerville park in Algoma, WI, and from Voyageur Park in De Pere, WI. We hypothesized the Voyageur Park location contained more microbes capable of antibiotic producers due to the location of the park being on a large, utilized river, and the larger surrounding population. We diluted, streaked, and plated our soil samples. Microbes found from our soil samples were placed on spread plates of *S. epidermidis* and *E. caratovora* to test for antibacterial producers. Microbes collected from Voyageur contained five antibacterial producers, while Bruemmerville contained one antibacterial producer. We then chose one antibacterial producer and plated it over relatives of ESKAPE pathogens. The bacteria from Voyageur Park had production against four ESKAPE pathogens. While the bacteria from Bruemmerville had production against two ESKAPE relatives. Our hypothesis was proven correct, showing the Voyageur sample contained more antibiotic-producing bacteria than the Bruemmerville sample. Our research on soil samples from Voyageur Park and Bruemmerville Park is still in progress, more results will be collected.

26 Cattails and Antibiotics: How an Antibiotic Producer was Found

Tracey Tanglin
Northeast Wisconsin Technical College

Our society relies heavily upon antibiotics to treat bacterial infections. However, since their introduction they have been overprescribed and misused leading to the rise of deadly strains of antibiotic-resistant bacteria. To combat current and future resistant bacteria, new antibiotics are needed. The Tiny Earth organization enables students to do hands-on research on soil in search of new antibiotic-producing organisms. To begin, a sample was collected from soil near a patch of cattails. My hypothesis was that there would be at least one antibiotic-producing bacteria found in my soil sample. The soil sample was diluted and inoculated on to agar plates. From there, 20 unique colonies were chosen and tested against *E. caratova* and *S. epidermidis*. Antibiotic activity was noted in two of the sample squares of the *S. epidermidis* plate. These results showed my hypothesis to be correct and show that there are antibiotic-producing bacteria at the site of the sample. Further testing is needed for identification.

27 Antibiotic Producing Soil Samples

Jessica Prescott
Northeast Wisconsin Technical College

Misuse of antibiotics in humans and animals has caused an increase in antibiotic resistant pathogens. Antibiotic resistance is rising at high levels in all parts of the world, threatening our healthcare, food production and life expectancy. The Tiny Earth Organization gave the opportunity for students to research soil in our communities to find new antibiotic producing microbes. Living in a rural community with agriculture heritage I decided to obtain a sample from what once was farming fields that is now a hobby farm with various animals. My hypothesis was that my sample would contain antibiotic producing microbes on account of the environment the sample was obtained from. The activity from different animals, wildlife and plant species made me believe that there would be a high number of microbes. I diluted and plated my sample. The microbes that were found were then plated and tested against *S. epidermidis* and *Erwinia* to see if they produced antibiotics. The bacteria had two possible antibiotic producers against *S. epidermidis*. I then chose one of the possible producers and screened against the ESKAPE pathogens, the results indicated that the sample produced against two additional pathogens. My hypothesis was correct with my sample containing antibiotic producing microbes. The research is still ongoing, results are to be determined.

28 Antibiotic Soil Collected from Prevea Health

Abigail Weber
Northeast Wisconsin Technical College

Antibiotic resistance, a topic many individuals know nothing about, has left scientists with a huge problem. The past few decades scientists have tried to find suitable antibiotics to aid in fighting against antibiotic resistant pathogens. The Tiny Earth organization gave our class the opportunity to research soil samples from our community. Searching for new microbes that will release antibiotics to fight against the harsh reality of antibiotic resistance. When I sat down and thought about global health and antibiotics the first thing that came to my mind was healthcare. Leading me to choose the location of the Prevea Heath Clinic to collect my soil sample. My hypothesis was that the Prevea Clinic soil would contain at least one microbe that generate antibiotic properties. I believed this to be an accurate hypothesis since I was collected soil from a clinic where an abondance of sick people go to receive medical care. I took the soil I collected from the Prevea clinic and diluted it. After diluting my soil, I took an agar plate and used the streaking technique. To find out which of the microbes would produce antibiotics, I took the microbes found and tested them against *S. epidermidis* and *Erwinia*. After the testing it came back that one spot tested against the two bacteria did have an antibiotic releasing microbe, in the 15th spot on the *Erwinia* plate. I chose that microbe to test against the ESKAPE pathogens. One spot came back that had some active clearing on it, spot 6 which had *E. Caratova* on it. My hypothesis was correct, I did have at least one antibiotic producing microbe. My research of the Prevea Health clinic soil is still on going, some of my results are still to be determined.

29 The Development of Antibiotic-Producing Microbes From Soil Samples of Wetland Locations

Halie Siudzinski, Kayla Bays, Brooke Smits
Northeast Wisconsin Technical College

The last antibiotic to be discovered was in the late 1980's. This is extremely detrimental in today's world because there are so many antibiotic resistant bacteria spreading. Due to antibiotic resistant bacteria, patients are no longer guaranteed effective treatment via antibiotics. This puts a heavy strain on scientists and the need for discovery of new antibiotics. We participated in the "Tiny Earth Project," in hopes we would discover a new antibiotic. We collected soil samples from three different wet land locations throughout Northeast Wisconsin. This included a drainage ditch, a neighborhood pond, and a wooded wetland. We predicted that a drainage ditch would produce the most antibiotic- producing bacteria due to the frequent movement of solutes. Once the soil was collected, we diluted the contents of each soil sample and spread on to LB growth plates. All our plates grew numerous amounts of bacterial colonies. We then each chose several unique colonies to experiment more with and all chose different conditions to grow the bacteria in. We then used the bacteria to test against *S. epidermidis* and *Erwinia carotovora*. The wooded wet land had two bacteria with antibiotic activity, the neighborhood pond and the drainage ditch had one bacteria with antibiotic activity. We then tested our bacteria against other relatives of known antibiotic resistant bacteria (ESKAPES pathogens.) These results showed that the wooded wetland and the drainage ditch had three areas of bacteria with antibiotic production. The wooded wetland had antibiotic production against *E. coli, B. subtilis,* and *P. putida*. The drainage ditch showed production against *E. coli, B. subtilis,* and E. *carotovora.* The neighborhood pond had no antibiotic production against the ESKAPES. Our results show that we were able to grow antibiotic resistant bacteria thus far. Therefore, we are on the road to discovering a new antibiotic.

30 Project: Isolate

Melinda Trinkner
Northeast Wisconsin Technical College

For decades, there have been known antibiotic-resistant pathogens that are making it difficult for physicians to treat infections, and less interest from pharmaceutical companies to do the research themselves in developing a new antibiotic. "The Tiny Earth Project" was developed for students to become a part of this solution in this crisis in hopes of finding new antibiotic-producing isolates from the soil they collected themselves. In selecting my soil, I chose to take from an old apple orchard where I believed there would be many promising microbes in developing a new antibiotic. The process started after collecting and diluting the soil and inoculating the sample on an agar plate, using the streak method then testing against the bacteria for *S. epidermidis* and *Erwinia* to see if my bacteria showed any activity against those organisms. I only had one isolate that was a double producer. I then chose one of my antibiotic producers and screened it against the ESKAPE pathogens. My results did not show any antibiotic producing microbes. My hypothesis was not accurate, since I only had one isolate that showed antibiotic activity and not several. This project is not completed yet and is ongoing. Not only for myself, but hopefully until there are several new antibiotics developed.

31 The Exploration of Soil-derived Antibiotic-producing Microbes Utilizing Avian Species

Kerin Thiry, Brittney Vlies
Northeast Wisconsin Technical College

The last antibiotic to successfully be introduced into society was in 1987. Antibiotic discovery is crucial in providing ways to rectify bacterial infections. To help scientists potentially discover a new antibiotic, we participated in the Tiny Earth Project, which allows students to aid in the research of antibiotic producing bacteria within the soil of our very own communities. We know soil holds many different microorganisms, so we then looked at what will carry these microorganisms and found that birds can obtain the bacteria and spread them throughout our communities. We collected soil from the Wildlife Sanctuary and from the chicken coop of our own home. We predicted that the Wildlife Sanctuary would contain more antibiotic producers within its soil due to the fact that the Wildlife Sanctuary is home to a variety of avian species as well as it being a public amenity to the community. Using the streak plate method, we inoculated our two samples from our communities against *S. epidermidis* and *E. carotovora.* The Wildlife Sanctuary contained two antibiotic producing microbes, while the sample taken from our chicken coop contained one antibiotic producing microbe. We then tested our samples against ESKAPE relatives. The Wildlife Sanctuary contained one antibiotic producing microbe, whereas the chicken coop sample contained two antibiotic producing microbes, with none of the microbes being dual producers. Our plates showed that both the Wildlife Sanctuary and the chicken coop sample contained three antibiotic producing bacteria, which proves our hypothesis incorrect, as we thought the Wildlife Sanctuary would contain more antibiotic producers.

32 Antibiotic Resistance and the Search for the Next Great Thing

Sara Stern
Northeast Wisconsin Technical College

The discovery of penicillin in 1920 by Alexander Fleming was a great advancement for the medical field and the health of humanity. Since then, there have been other antibiotics that have proved to be just as useful. But nothing new has been discovered since 1980 and each year the cases of antibiotic resistant infections continue to increase. The Tiny Earth project was started to help fight this problem. Students from around the world are isolating bacteria from soil samples in hopes of one day finding the next great antibiotic. I collected my sample from the corner of a field on my farm. Over the years there has been manure piled there and one year volunteer pumpkins sprouted on their own. It is also next to a waterway and across the lane from the cow pasture. My hypothesis was it would be a good environment for antibiotic producing microbes because the soil has been undisturbed for many years. The pure culture extracted from my soil sample was first tested against *S. epidermidis* and *E. carotovora* and since the sample was taken quite deep and from a damp area, I chose the medium that did well in those environments. My sample showed no antibiotic activity against either of those organisms. Next it was placed on plate to see if it would show resistance to relatives of ESKAPE pathogens. After 24 hours and one week it showed no activity against the other microbes. My hypothesis has not been correct. Currently, the bacteria show no antibiotic producing capabilities. As we continue with our research, I hope to be able to identify my bacteria and find antibiotic producing qualities from it.

33 Digging for Antibiotic Producing Bacteria

Kiarra Kubinski
Northeast Wisconsin Technical College

In class, we discussed research that estimated 10 million deaths in the United States would occur each year by 2050 due to antibiotic resistance. According to the CDC, more than 2.8 million antibiotic-resistance infections occur annually in the United States. It takes about ten years to produce an antibiotic, and scientists cannot keep up. That is why NWTC has partnered with Tiny Earth organization to help fight the health crisis of our decreasing supply of effective antibiotics from misuse. Our class has divided into groups to do investigative research on antibiotic-producing bacteria by taking a deeper dive into the bacteria living in the soil. I chose to retrieve my soil from a local school playground (Little Chute Elementary) due to the amount of daily activity school playgrounds receive 9 months out of the year. A survey conducted by The Global Hygiene Council (GHC) found that children do not follow proper hand-washing techniques in school. This number of germs and bacteria is most likely to rapidly distribute with the activities children choose to engage in their daily living activities, such as roughhousing during recess. I hypothesized that LC elementary would contain antibiotic-producing bacteria in the soil. The microorganisms in our soil were isolated using a series of dilution methods and then plated where we waited for our bacteria to grow. After isolating the bacteria, we chose many unique colonies to test against *E. coli,* and *Staphylococcus* to see which microbes produce antibiotics against them. My bacteria had one dual producer, so I chose to continue testing on that bacterium. Once an antibiotic-producing colony was revealed, they were further tested against 7 different ESKAPE pathogens to determine if they had antibiotic-producing qualities against these pathogens and to help identify them. My results were positive against one of these which proved my hypothesis accurate of finding an antibiotic-producing bacteria in Little Chute elementary soil. There is further testing in progress therefore some results are yet to be determined.

34 Tiny Earth: The Fight Against Super Bugs

Marcus Westrich
Northeast Wisconsin Technical College

Current antibiotics are losing their effectiveness against microbes, and those same microbes are evolving and becoming more resistant to the medications we use to treat them. Soon, we may be facing waves of pandemics of highly resistant microbes with little to no way of treating them, this is what Tiny Earth is meant to combat. Microbiology classes from numerous different colleges are putting their students to work to not only educate, but to also maybe find new forms of antibiotics that we can use against these resistant microbes. We do this by collecting soil from all over in numerous different environments, isolating colonies of bacteria in our labs, and testing their antibiotic capabilities against related, yet safer, strains of the most common resistant bacteria. My hypothesis was that I would find at least one kind of unique bacteria that showed antibiotic production. I was interested in searching the soil of NWTC campus, not only for the purpose of trying to find possible antibiotic bacterium and prove my hypothesis, but also to identify microbes there may be on my own school grounds. There were very few kinds of unique bacteria I found from my sample when grown on petri dishes, only one or two, both showing not much antibiotic behavior. Further tests will be completed to determine what type of bacteria is present.

35 Antibiotic Producer Collected by the East Twin River

Erin Wachowski
Northeast Wisconsin Technical College

Antibiotic resistant pathogens have been a challenge for many scientists. Tiny Earth has involved students in the research to find new microbes found in soil that can be antibiotic producing. For my Tiny Earth project I took a soil sample from my backyard. I live on the river where there is a marsh on the edge of my yard. My hypothesis is that there will be microbes that are antibiotic producing, because there is a high number of animals ranging from deer, birds, waterfowl, and fox that are always in the backyard. Also the soil contains more water and the temperature of the soil seems to be warmer. I think that in an environment like this it gives microbes more opportunity for survival and growth. I diluted my soil sample and plated the diluted samples by using the streak plate method. Out of all the microbes I collected I had one that after twenty-four hours showed potential activity for antibiotic production to both *S. epidermidis* and *Erwina*. Then I chose that microbe and placed it on a LB plate with ESKAPES relatives. After 24 hours five out of eight had show production. My hypothesis is accurate because from my soil sample I have a microbe that has potential to be an antibiotic producer.

36 Tiny Earth: Antibiotic Discovery Research

Christian DallaSanta
Northeast Wisconsin Technical College

Raising awareness and educating on antimicrobial resistance is a topic neglected around the world. In 2019 the CDC reported antibiotic resistant infections reaching 2.8 million- leading to 35,000 deaths a year in the United States. This presentation will display my efforts in attempting to discover a new antibiotic microbe to help save lives.

37 Antibiotic Producers Collected from Duck Pens and Cow Pastures

Danielle May, Amy Guenther, Lisa Reinke, Kailyn Voskuhl
Northeast Wisconsin Technical College

Antibiotic resistance has started to become a global problem in the last few decades, significantly increasing the mortality rate. The Tiny Earth Organization has reached out to us to help them find new antibiotics from the soil in our communities. We took soil samples from two duck pens and two cow pastures. Our hypothesis was that the duck pens will contain more antibiotic producing bacteria because ducks travel farther and could possibly get contaminates on their feathers. We believe that the soil we collected from the pens/pastures will contain a lot of bacteria, because bacteria in soil exists near areas that have animals living. Therefore, it is making our soils enriched with antibiotic agents. All four soil samples were collected using the Tiny Earth research procedures. We first took our soil samples and diluted them. Next, we used a grid pattern to plate them, and eventually used the streak plate technique, which is used to isolate just one colony type of bacteria. The microbes that grew from those plates were then inoculated and screened against the bacteria *S. epidermidis* and *Erwinia* to see if any of the microbes would produce antibiotics against the bacteria. The cow pastures had five antibiotic producing microbes while the duck pens had only one antibiotic producing microbe. Our hypothesis was not accurate, as our cow pastures had more antibiotic producing microbes. Some testing and results are still ongoing and yet to be determined.

38 Collaborations in Tiny Earth: A Synthetic Approach to Antibiotic Discovery

Amber Burzych
Northeast Wisconsin Technical College

Antibiotic resistance is a growing problem, leading to the development of projects like Tiny Earth to search for new antibiotic producing bacteria. The microbiology courses at NWTC have been participating in Tiny Earth for several years, and recently, students in Organic Chemistry 2 have been participating in an extension of Tiny Earth. In collaboration with the biology team, organic students participated in mini research projects to synthesize and test molecules for antibiotic activity using protocols from Tiny Earth. Students synthesized small libraries of N-acyl derivatives of amino acids using solid phase synthesis. These compounds were then purified and screened for antibiotic activity. Through this process, several compounds showed potential, and current students are continuing to further investigate these compounds.

39 The NWTC Trail

Savannah Rosenberg
Northeast Wisconsin Technical College

The Tiny Earth Project inspires Microbiology students across the nation to aid in the discovery of bacteria that can produce antibiotics. Students are recruited to take soil samples to search for new antibiotics, which could positively benefit our healthcare systems given the current amount of antibiotic resistant bacteria. I chose to take my sample from a place that is relatively untouched, but close to home. My hypothesis was that there may be helpful microbes that produced antibiotics in the soil behind NWTC because it was damp, cool, and it did not appear that there had been much activity from humans or animals. After using water to dilute my soil and isolating bacteria, I tested it against *E. carotovora*, as well as *S. epidermidis.* After waiting several days, I checked to see if there was any antibiotic activity. On one of my plates, I discovered that there was a clearing on several spots. This means I found microbes producing antibiotics toward *Erwinia*. After also checking reactions against the ESKAPE relatives sometime later, I realized I had not found any unique antibiotic producing bacteria, which means my hypothesis was incorrect. I had several similar colonies on one plate, but close to nothing on the other. Changing the location of my sample or the type of plate I used to test could be helpful in improving my results.

40 Analysis of Soil From a Farm in Seymour

Olivia Montie
Northeast Wisconsin Technical College

Antibiotic resistant pathogens are a growing concern for scientists, and even more so because of the demand to find new antibiotics. This in turn poses a threat to the health of the population; without effective antibiotics, people can die from simple infections. The Tiny Earth Organization opened the door for students to discover new antibiotics. I believed that finding an area with the most unique microorganisms would increase my chance of discovering an antibiotic producing bacteria, so I choose a dairy farm. My hypothesis was that my site would contain at least two antibiotic producing species. I diluted my soil and put them on LB plates using the streak plate method, this allowed me to compare my different colonies. However, this didn't give me a whole lot of variety, so I changed the media. Since my soil sample was moist, I decided to use R2A as one of my media's. I also used TSA for a different media because it supports growth for a wide variety of organisms. To test my colonies for antibiotic production, I tested them against *S. epidermidis* and *Erwinia*. I then took the antibiotic producing bacteria and tested it against relative ESKAPE pathogens. This will show me if it is effective against any of the other relative pathogens using the same method as previous. My microbe ended up displaying antibiotic activity on *E. coli.* Overall, my microbe was effective against two different pathogens. So, my hypothesis was incorrect, but I still have an effective microbe. I am still researching and testing my soil sample from the dairy farm, so some results are yet to be determined.

STUDENT POSTERS

41 Farm Fields and Antibiotic Discovery

Kari Grall, Joanie Wudtke, Brooke Kelly
Northeast Wisconsin Technical College

Every year in the United States, millions of people are infected with an anti-resistant bacterium. When antibiotics are overprescribed and improperly used, antibiotic resistance occurs. The amount of antibiotics being discovered today has significantly decreased. To be exact there has not been an antibiotic discovered since the 1990s. Our Microbiology class is participating in the Tiny Earth Project. This network was created by Doctor Jo Handelsman to find new antibiotics. This is done by isolating bacteria from soil and screening it to identify those that produce antimicrobials. Our group collected soil from corn, soybean, and a hay field. We then diluted our soil samples and grew bacteria on agar plates. The goal was to find isolated colonies of bacteria so that we could test them for any antibiotic-producing capabilities. To do this, we took twenty different colonies of bacteria from each soil sample and grew it on a plate to see if any had the capability for antibiotics. We used the ESKAPES test to see if our samples had any antibiotic-producing capabilities. The soybean and hay samples had zones of inhibition; the corn sample did not have any inhibition. The three of us only had one each that produced antibiotic properties. We then used the ones with these properties and ran them through PCR and biochemical testing. We hope to discover antibiotic compounds from our soil samples that are not already known in hopes of finding new ways to fight bacteria.

42 The Biodiversity between Northern and Eastern Wisconsin

Madeline Dorn, Raven Schuette
Northeast Wisconsin Technical College

With the discovery of antibiotics slowing to a crawl, over-prescription, and break-out microbes, the need for new antibiotics has never been higher. The threat of multiple-antibiotic resistant organisms is an epidemic that needs to be addressed. The Tiny Earth project aims to help combat this by involving students and communities in the fight to discover new species of antibiotic producing microbes. We took two samples of soil, one from Northern Wisconsin, and another from Eastern Wisconsin. We hypothesized that the Northern Wisconsin sample would be more likely to have antibiotic producing bacteria because it appeared to be more suitable for bacteria due to a more nutrient rich ground with shade above it to promote microbe growth. We started the process by diluting and streak plating both samples on three different types of streak plates to increase the variety of the microbes produced. We chose twenty different bacteria from each sample to continue with and plate. From there we incubated and chose one bacteria from each sample based on the highest likelihood of having antibiotic capabilities against relatives of the ESKAPE pathogens, which are some of the multiple-antibiotic resistant organisms we need to fight. The Eastern Wisconsin sample had two potential antibiotic producing bacteria against *S. epidermidis* and *A. baylyi* while the Northern Wisconsin sample had one potential producer against *E. coli.* While our hypothesis may have been for the Northern Wisconsin sample to have more producers, it turns out that the Eastern Wisconsin sample had more. Our research is still on going to determine if we have found a species previously found, or if we have helped find a new helper against these multiple-antibiotic resistant microbes.

43 Antibiotic Producers Collected from a Marian, WI Farm

Emily Plaster
Northeast Wisconsin Technical College

Tiny Earth is allowing us to research soil in our communities. I collected my soil sample from a small farm in Marian Wi. Soil was collected on 9/10/2022 at a depth of 1 foot, soil was determined to be clay-like soil. The temperature when the soil was collected was 58 degrees and the weather was rainy. The purpose of this experiment is to see if the soil has produced new microbes that release antibiotics. My hypothesis is that my soil would produce microbes with antibiotic properties. I came to this conclusion since this farm has been worked in several different ways for over 100 years. Having several different operations over the past 100 years the microbes would be able to multiply more easily. we diluted our soil sample and plated different concentrations; I chose a plate with seven unique colonies. After completing this we chose 2 separate plates to change the variables – I chose Pda and TSA plates. With my new plates, Pda produced several different colonies while the TSA plate only produced one colony. To see if our microbes would produce antibiotics, we placed them on three separate plates and spread the following; *S. epidermidis, E. carotovora*. After incubation we observed which plates had antibiotic clearing, I had antibiotic clearing on both plates in area number one which was a circular smooth bacterium from plate 4. I completed a 4-phase streak plate with my chosen bacteria and created an all-tester plate – we then inoculated this plate with ESKAPES. My bacteria had additional production against *E. coli, Bacillus, Acinetobacter, Erwinia,* and *Enterobacter*. Colonies that were produced were yellow and circular with rough edges. We ran PCR and gel electrophoresis, and the product was sent off for sequencing.

44 Tiny Earth 2022

Megan Holz
Northeast Wisconsin Technical College

Many bacteria that cause disease are becoming resistant to current antibiotics that are available. I predict that the area chosen for my soil collection will have bacteria with antibiotic properties.

I obtained soil from a preselected location; I chose to obtain soil from the opening of woods on our property as we do not use pesticides or herbicides anywhere near there. As well as it is farthest from the field next to us. The sample was diluted and placed on media plates to grow bacteria in the soil. Picking different unique colonies, we placed our chosen organisms into a *Staph (S. epidemidis)* plate, *Erwinia (E. carotovora)* plate, and on a master plate to determine if the bacteria chosen had effects against the determined bacteria.

I determined that my organism chosen had shown resistance to some of the ESKAPE bacteria. It showed antibiotic properties against *Acinetobacter* and *Enterobacter* bacteria. My experiment is ongoing. I hope to have evidence that my organism has potential to become a new antibiotic.

45 Antibiotic Producing Bacteria on the Wolf River

Grace Solveson, Bayleigh Laabs
Northeast Wisconsin Technical College

Recently pathogens have been slowly building immunity to antibiotics which means bacterial diseases may become untreatable until new antibiotics are discovered. Tiny Earth has the goal of engaging students in original research by attempting to discover new antibiotics. The majority of our antibiotics we use today have come from bacteria found in soil. For our Tiny Earth lab, we collected two soil samples from two different locations along the Wolf River. We chose the Wolf River because it has a diverse, moist ecosystem which is the perfect environment for growth of many different antibiotic producing bacteria. For our soil extractions we thought that comparing two different locations will result in finding different antibiotic producing bacteria between the sites. We ran several tests on our samples in the NWTC microbiology lab. The same tests were run for both extraction sites. The samples were diluted, plated on culture slides, and tested against *S.epidermidis* and *Erwinia*. The sample from the first location had three possible antibiotic releasing microbes while the sample from the second location had one possible antibiotic releasing microbe. We each chose one possible antibiotic releasing microbe to use for a master plate and all tester ESKAPES tests. By using these tests we were able to discover and compare antibiotic activity from two different locations along the Wolf River. We found that bacteria from both samples grew extremely well in the dark and when grown on R2a nutrients. The sample from the second location plates did not show any activity with the ESKAPES tests, but the sample from the first location plate showed some antibiotic activity with clearing against E.coli. Our results showed that while bacteria from both sites thrived in the same growing conditions, one site may potentially have more antibiotic producing bacteria than the other site. This supports our hypothesis that soil extracted from different locations on the Wolf River contained different antibiotic producing bacteria. We are still discovering different findings within our experiment.

46 Antibiotic Bacteria

Alyssa Spagnoletti
Northeast Wisconsin Technical College

As a class we set out to find bacteria in various places that could potentially have antibiotic properties. By doing this project we hope to find new bacteria to make new antibiotics that are much needed as world has an ongoing problem of antibiotic resistance. My project was obtaining a sample from my woods by the Suamico river, to find bacteria containing antibiotic properties. The purpose is in finding an antibiotic producing bacteria. My hypothesis is that this site will produce 1 of antibiotic producing species. The methods I used was using the soil collection kit to dig a spot and collect my sample. Then I diluted the soil sample and grew bacteria plates, to create at least one bacteria colony. Next, I tested different growing conditions, like incubating in "the" dark and using PDA media. Using the master plate and tester, I tested my bacteria all on an LB plate. I first tested my chosen bacteria for production against *S. epidermidis* and *Erwinia*, then against other ESKAPE relatives. Next, I ran PCR and gel electrophoresis. My experiments so far have shown that my bacteria like dark and PDA environment, it did not show activity against *S. epidermidis* and *Erwinia* for one test but not on the all-tester plate it had no antibiotic reactions with the ESKAPES pathogens. My bacteria was positive for 16s r RNA gene and showed one band on the gel electrophoresis. My results show that I could have some antibiotic producing capabilities and my bacteria has 16s rRNA gene to help me figure out a DNA sequence.

47 Antibiotic producing bacteria found in compost

Krystle Cretens
Northeast Wisconsin Technical College

We have made great strides in medical research and development since 1928, when the first antibiotic producing bacteria was discovered. Unfortunately, due to antibiotic resistant strains of bacteria we are still losing lives. This could be combated if we were to find new antibiotics that could kill the resistant bacteria. This is the reason for the research that we are conducting. I took my soil sample from a compost pile. The reasoning behind my decision was that there is a lot of different looking molds in the pile and that molds were how the first antibiotic was discovered. My hypothesis was that I would find at least one antibiotic producer. During diluting the soil sample taken and testing it against many different types of known antibiotic resistant bacteria, I found two antibiotic producers. My hypothesis was correct in the fact that I would find at least one antibiotic producers. I have chosen one bacterium that showed the most clearing when tested against known antibiotic resistant bacteria. The results of the name of my bacteria are still on going and I am awaiting result from UW Madison.

48 Which Lake is Superior?

Kala Notz, Alex Perleberg
Northeast Wisconsin Technical College

Antibiotic resistance is a significant global health problem. Bacteria and other pathogens are adaptable and can change their genetics to resist the effect of medications. The Tiny Earth Project is looking to help, by having students collect soil samples and look for antibiotic producing microbes while in Microbiology classes. We gathered soil samples from the Egg Harbor Beach located in the Bay of Green Bay and the Jacksonport Beach next to Lake Michigan in Door County. Our hypothesis was that the soil sample collected in Egg Harbor would produce more antibiotic producing microbes. This is due to the size of the Bay vs the Lake and that the temperature of the water there has tended to be much warmer which produces a better environment for growth. We used the soil samples in lab after diluting it down and placing it on many different types of media that could assist in the rapid growth of our microbes. We used individual pure colonies and placed them on our plates of *S. epidermidis* and *Erwina* to see if there were any antibiotic producers. We each had one bacterium that showed activity from Egg Harbor and Jacksonport on the *S. epidermidis* plate.

49 Antimicrobial Effects of Commercial and Novel Compounds

William Butak
St. Norbert College

The antimicrobial effectiveness and fungal spore inhibition of ColaLipid (CL) – a surfactant used by Rockline Industries – was tested, along with the antimicrobial effectiveness of sixteen compounds made by the St. Norbert College Organic Chemistry Department. While CL is soluble in water, the sixteen compounds (primarily indoles and surfactants) need to be solubilized in 10% DMSO. All compounds were tested against eight bacteria, each of which are safer relatives of clinically significant bacterial strains. The CL was also tested against nine species of fungus to test the inhibition of fungal spores. Microbial growth was measured by measuring the absorbance of overnight cultures. ColaLipid was found to strongly inhibit growth of the bacterial and fungal strains, as well as limit fungal spread by eliminating viable spores. Of the compounds synthesized by St. Norbert organic chemists, three showed some inhibition of growth, which can be explored and expanded on in future work.

50 Exploration of endophytic species from Wisconsin towards the discovery of new natural product antibiotic scaffolds

Katie Garber, Lilia Shallow, Collin Sylvain, Samantha Pardini
St. Norbert College

To locate potential novel natural product antibiotics, we are focusing on endophytes, bacteria and fungi that colonize the interior organs of a host plant without causing pathogenic effects. The unique plant-endophyte relationship is maintained by a complex secondary metabolome that has been evolutionarily optimized for biological efficacy. We hope to exploit the paucity of information regarding endophytes in Wisconsin to maximize our potential of finding novel organisms and, subsequently, novel antibiotic scaffolds. We have focused on plants from unique local ecosystems, that are endemic, that are known to have an unusual longevity, or that have a reported ethnobotanical history; these types of plants are thought to be most likely to harbor a diverse set of endophytic organisms and, therefore, a diverse set of bioactive natural products. The endophytes isolated from our plant samples are co-cultured with several bacterial pathogens to screen for production of secreted secondary metabolites with antibiotic activity.

51 In the Wild: Antibiotic Discovery

PJ Schneider, Jarod Witt
UW-Green Bay

Antibiotic resistance is a global health crisis. The international Tiny Earth research effort explores soil samples for antibiotic-producing bacteria to mitigate the health emergency. A soil sample was collected on the University of Wisconsin – Green Bay campus (44.52575N, 87.9191W). One gram of soil was diluted in water and plated on Luria (LB) agar plates. Antibiotic activity was tested by patching isolated soil bacteria on Potato Dextrose agar (PDA) and LB agar plates containing either *E. coli, S. epidermidis,* or *B. subtilis.* Antibiotic activity was observed in four soil isolates.

52 Discovery of Antibiotic-Producing Bacteria

Morgan Gottowski, Mason Zeise
UW-Green Bay

Antibiotic resistance is a global health emergency. The Tiny Earth Project aims to discover new antibiotic-producing bacteria from the soil. A soil sample was collected from the University of Wisconsin – Green Bay campus (44.52958O N, -87.92042O W). One gram of the sample was diluted in water and plated on Luria broth (LB) containing cycloheximide and incubated at 28 Celsius for 48 hours. Master plates were created by picking and patching isolated bacteria to Potato Dextrose agar (PDA) and LB agar plates. Antibiotic activity in each isolate was determined by patching soil isolates on PDA and LB agar plates containing either *Staphylococcus epidermidis, Bacillus subtilis (B. subtilis)* or *Escherichia coli (E. coli).* Four isolates displayed antibiotic activity overall: two of our isolates showed antibiotic activity against *E. coli* on PDA plates, and three isolates showed antibiotic activity against *B. subtilis* on LB agar plates.

53 Antibiotic Discovery

Alan Vang, Katherine Drews
UW-Green Bay

Tiny Earth is a global research curriculum that provides opportunities for students to take an active role in real-world scientific research to address the world crisis of antibiotic resistance by studying antibiotic activity by soil bacteria. The soil was collected on the UW-Green Bay campus GPS coordinates (44.5299399, -87.9228293) to assess the role of vegetation, heavy foot traffic, and soil erosion, on antibiotic production as determined in the lab. One gram of soil sample was diluted in water and plated on Luria Broth Agar (LB) plate with cycloheximide to isolate bacteria. The antibiotic activity was determined by patching soil isolates to LB and Potato Dextrose Agar (PDA) containing either *Bacillus subtilis (B. substilis), Escherichia coli (E. coli)* or *Staphylococcus epidermidis (S. epidermidis)*. One isolate displayed antibiotic activity against *B. substilis* and *E. coli* on both LB and PDA.

54 Soil Analysis to Discover Antibiotics

Daniel Powers, Liz Kubica
UW-Green Bay

Antibiotic-resistance is a public health emergency. Tiny Earth is an international research program to discover novel antibiotics produced by soil bacteria. A soil sample was collected on the UW-Green Bay campus (44.52654 N, 87.92656 W) based on the surrounding environment and resident vegetation. One gram of soil was serially diluted in water and placed on Luria Broth (LB) agar with cycloheximide. Soil isolates were patched to both Potato Dextrose Agar (PDA) and LB agar containing either *E. coli, B. subtilis,* or *S. epidermidis* as test strains to evaluate antibiotic activity. It was found that four isolates displayed antibiotic activity against *B. subtilis* on PDA agar.

55 Antibiotic Soil Collection

Pa Kou Yang, Jayna Salvinski
UW-Green Bay

A soil sample was taken from a site on the UW-Green Bay campus previously shown to harbor antibiotic-producing bacteria near the Mahon woods of the Cofrin Arboretum (44.526546l -87.910182). One gram of soil was serially diluted in water and plated on Luria Broth (LB) agar plates containing cycloheximide. Isolates were patched on PDA and LB agar plates to create master plates. Antibiotic activity was evaluated by patching isolates to PDA and LB agar plates containing either, *S. epidermidis, B. subtilis,* or *E. coli*. One isolate demonstrated antibiotic activity against *B. subtilis* on LB agar.

56 The Secrets of the Soil

Nicholas Kohlmann, Alexandria Zakutney
UW-Green Bay

In 2019, approximately 1.27 million people died as a result of infections caused by antibiotic-resistant bacteria. The goal of this research is to discover antibiotic-producing bacteria in the soil to mitigate the crisis. A soil sample was collected on the UW-Green Bay campus (44.52990°N, 87.93375°W). This sample was diluted in water and plated on four Luria Broth (LB) agar plate containing cycloheximide. Fifteen isolates were patched to Potato Dextrose Agar (PDA) and LB agar to create master plates. Antibiotic activity was evaluated by patching isolates to PDA and LB agar containing either *E. coli, S. epidermidis,* or *B. subtilis.* Of the fifteen evaluated, five isolates demonstrated antibiotic properties against *B. subtilis* on PDA and LB agar: One isolate showed activity against *E. coli* on LB agar.

57 On the Hunt for Antibiotic Producers in UW-Green Bay Soil

Anthony Williamson, Sammy Stevenson
UW-Green Bay

A major public health issue is the increasing number of infections caused by antibiotic-resistant bacteria. The Tiny Earth initiative provides opportunities for students to identify novel antibiotics produced by soil bacteria. A soil sample was collected from the UW-Green Bay Campus in a high-traffic area (44.52698°N, 87.92631°W). One gram of soil was diluted in water and plated on Luria Broth (LB) agar plates containing cycloheximide. Isolated bacteria were picked and patched on LB agar and Potato Dextrose agar (PDA) plates. Antibiotic activity was tested by patching soil isolates on PDA and LBA agar plates containing either *Bacillus subtilis, Escherichia coli,* or *Staphylococcus epidermidis.* Three isolates displayed antibiotic activity against *B. subtilis* and *S. epidermidis.*

58 Soil Pit Bacteria

Haley Herwald, Erin Witt
UW-Green Bay

Tiny Earth is a global research effort to address the global health crisis of antibiotic-resistant bacteria.

Soil was collected from the A horizon on the UW-Green Bay campus (44.53247° N, 87.9276°W). The site was chosen because it is frequently accessed and used by UW-Green Bay students and instructors. One gram of soil was serially diluted in water and plated on Luria Broth agar (LB) and Potato Dextrose Agar (PDA) plates containing cycloheximide, generating 4.9e6 CFU/g. Isolates were evaluated for their antibiotic activity against *Bacillus subtilis, Staphylococcus epidermidis*, and *Escherichia coli* on PDA and LB agar. One of the nine bacterial isolates displayed antibiotic activity against *Staphylococcus epidermidis* on PDA

59 Antibiotic Discovery

Dannaley Krenz, Ben Sollberger
UW-Green Bay

The world faces difficulties treating infections caused by antibiotic-resistant bacteria. Tiny Earth is an international community of student scientists engaging in antibiotic research and discovery. A soil sample was collected from the UWGB campus near areas of vegetation in low lying areas (44.530181, -87.92007). One gram of soil was serially diluted in water, then plated to Luria Broth (LB) agar plates containing cycloheximide for 48 hours at 28 °C. The isolated bacteria were picked and patched onto potato dextrose agar (PDA) and LB agar plates containing either *E. coli, S. epidermidis* or *B. subtilis* to evaluate antibiotic activity on these test strains. Three isolates displayed antibiotic activity against *B. subtilis* on PDA plates.

STUDENT POSTERS

60 Antibiotic-producing Soil Bacteria

Andrew Duncan, Kaleb Voight
UW-Green Bay

Antibiotic-resistance is a world crisis. Tiny Earth is an international research project to identify novel antibiotics produced by soil bacteria. Soil was collected on the UW-Green Bay campus (44.53031° N, 87.92370° W). One gram of soil was serially diluted in water and plated on Luria Broth (LB) agar plates containing cycloheximide. Nine colonies were patched to LB agar and Potato Dextrose Agar (PDA) as a source of material for additional tests. Antibiotic activity was evaluated by patching soil isolates to PDA and LB agar plates containing either *Escherichia coli (E. coli, Staphylococcus epidermidis (S. epidermidis)* or *Bacillus subtilis (B. subtilis)*. Six isolates displayed antibiotic activity against *B. subtilis* on LB and PDA agar plates.

61 Using Microsoft Azure and Machine Learning to improve the efficiency of a global effort to discover novel antibiotics in soil samples

Brian Merkel, Iftekhar Anam, Chris Houghton
UW-Green Bay

The Tiny Earth initiative is a global student-sourcing research effort embedded in college laboratory courses to identify new antibiotics produced by soil bacteria. The goal of this project is to build predictive models to identify soil environments harboring bacterial isolates producing novel antibiotics of interest. We developed a model that could predict whether we could find soil bacteria that produce antibiotics that display narrow or broad-spectrum actvity against safe relatives of ESKAPE pathogens.

62 The End of a Crisis?

Caitlyn Pingel, Johannah Duncan
UW-Green Bay

More and more bacteria are becoming resistant to antibiotics. Not only are the bacteria becoming more resistant, fewer companies are investing in antibiotic discovery. One gram of soil was obtained from the UW-Green Bay campus at 44.52686°N, -87.90874°W, near the presence of a puffball mushroom. The soil was then serially diluted in water and plated on Luria Broth Agar (LBA) with cycloheximide. Isolated bacteria were patched on potato dextrose agar (PDA) and LBA to create master plates. Antibiotic producers were then evaluated by spread/patch technique using PDA and LBA plates. *Escherichia coli* and *Staphylococcus epidermidis* were used as test strains. Five isolates displayed antibiotic activity.

63 Antibiotic Resistance Beneath our Feet

Isis Sorto Matute
Green Bay West High School

Tiny Earth is a program that studies antibiotic discovery by soil bacteria and the crisis of antibiotic-resistant bacteria. Soil samples were collected on the UW-Green Bay campus (44.52943*N,87.91909*W.) After collection, samples were serially diluted onto Luria Broth (LB) agar plates. Based on the number of colonies, colony forming units per gram (CFU/g) were calculated to be 5x10^10. Then, the isolates were picked and patched to create master plates. The isolates were then patched to PDA and LB plates containing either *Escherichia coli (E.coli)* or *Bacillus subtilis (B. subtilis.)* Any inhibition of growth was considered an indication of antimicrobial activity. Out of ten isolates that were tested, five isolates showed activity against a variety of test strains on different media.

64 Discovering Antibiotic Producers, One Gram of Soil at a Time

Abigail Nowak, Andrew Kroll
UW-Green Bay

The development of new antibiotics is dwindling. A soil sample was collected at the University of Wisconsin Green Bay (-44.53007 °N, 87.92056 °W) near undisturbed soil with surrounding vegetation. One gram of soil was serially diluted in water and plated on Luria Broth (LB) agar containing cycloheximide and incubated at 28°C for 48 hours. 1.3*10^6 colony-forming units per gram (CFU/g) were isolated. Antibiotic activity was determined by patching soil isolates on LB agar and Potato Dextrose agar plates containing either *Escherichia coli* or *Bacillus subtilis*. Five isolates demonstrated antibiotic activity against *Bacillus subtilis* on PDA and LB agar plates.

STUDENT POSTERS

65 The Discovery of Microscott

Gabriella Urbano, Sophie Franzmeier, Adriana Gaitan, Lisa Sulla
Carthage College

Identifying new antimicrobials is needed due to pathogenic antibiotic resistance increasing. The Tiny Earth Project provided us with the opportunity to confirm and test our isolated bacteria for research in hopes of finding an antibiotic-producing bacteria. One of our isolates was obtained from Lake Michigan using FW70 media, and the others were isolated from soil on the Carthage College campus using serial dilution on DNA agar media. Isolates were tested for antimicrobial activity on, *Acinetobacter baylyi* and *Enterococcus*. Our bacterial isolates had antimicrobial activity against ESKAPE relatives of pathogenic bacteria and were obtained in pure cultures. Gram staining indicates that they are all gram-negative. Thioglycolate assays indicate that one of the soil isolates is an aerotolerant anaerobe and the others are obligate aerobes. The water isolate is a facultative anaerobe. Further biochemical tests and 16S rRNA sequencing are being performed to characterize and identify each species of bacteria.

66 Isolation and Identification of Microorganisms from Lake Michigan and Surrounding Soil

Cecilia Curran
Carthage College

Identification and isolation of new antimicrobials is needed to combat the rise of antibiotic resistance. As part of the Tiny Earth project to find new antimicrobials, water samples were collected from Lake Michigan and isolated using R2A plates, while soil samples were collected near the Pike river on the Carthage College campus and isolated using TSA plates. Isolates were tested on ESKAPE relatives of pathogenic bacteria, and four isolates that showed antimicrobial activity were obtained in pure culture. Isolate #1 had antimicrobial activity against Enterococcus faecalis, #3 had activity against *Acinetobacter baylyi*, and #5 and #7 affected *Mycobacterium phlei*. Staining and thioglycolate assays indicate isolate #1 is an obligate aerobic, gram positive rod, while #3 and #7 are facultative anaerobic, gram positive rods. Isolate #5 is a facultative anaerobic, gram negative rod. Further biochemical tests and 16S rRNA sequencing are being performed to identify and characterize each species of bacteria.

67 Tiny Earth Project: Garden

Kaitlynn Springborn
Northeast Wisconsin Technical College

Did you know that the last antibiotic class was discovered in 1987? With hundreds of antibiotics out on the market we are still facing something known as antibiotic resistance. Antibiotic resistance is when bacteria have the ability to survive the antibiotic that is supposed to kill them. The Tiny Earth Project is working with schools all around to find new microbes that have antibiotic properties by having students collect a soil sample from a location of their choice. For my project I chose to take a soil sample from my parents' garden. My hypothesis is that the soil contains many bacteria that have antibiotic properties because of the fallen fruit and vegetables that had fallen and decomposed throughout the summer and the manure used to fertilized the garden. After collecting my sample, I diluted my soil and transferred the dilution onto different types of agar and put them in different environments to see if different types of bacteria would grow. The next step was to transfer twenty different colonies from those plates onto three different plates. One was a master plate that was just the colonies, one plate inoculated with *S. epidermidis,* and one with *Erwinia*. Around some of the colonies I saw clearing, which demonstrates antibiotic properties. I picked one of the bacteria that looked like it had the most antibiotic properties and made a streak plate out of this bacterium and then put it to test against eight different ESKAPE relatives. This showed my bacteria produced against those seven bacteria. In conclusion my results showed that my hypothesis was correct and that I had found a bacteria that has antibiotic properties. I still have some experimenting to do with my bacteria and am excited to share my final results with you!

68 Antimicrobial Screening of Bacterial Isolates

Matthew Gille
St. Norbert College

Investigation into the antimicrobial activity of 22 isolates of unknown identity against eight bacterial species was conducted. Previous lab work at St. Norbert College had flagged these isolates as potentially producing antimicrobial products. Isolate samples were streaked on MH plates and allowed to grow simultaneously with a lawn of the competing bacteria. Inhibition was measured by observing a zone of inhibition around streaked isolates. Three of the 22 isolates (isolates 1, 10, and 22) showed particular signs of inhibitory activity against the Acinetobacter genus following preliminary assays. The other 19 isolates failed to display clear signs of inhibition against any of the competing bacterial species. Isolate 1 produced readable sequence following 16S rRNA PCR, and BLAST analysis revealed it to be in the genus Chryseobacterium. Moving forward, secondary metabolites produced by isolate 1 will be extracted to determine their chemical identities.

69 Isolation and Identification of Potential Antibiotic-Producing Bacteria

Olivia Peplinski, Kayleigh Carotenuto, Kalene Koziczkowski, Emma Rabidoux
St. Norbert College

With bacteria's increasing resistance to a wide variety of our most commonly employed antibiotics, we have no choice but to focus our energy on discovering new treatments against bacterial infections. Working with the Tiny Earth Project, bacteria (8.8x10^5 CFU/mL) were isolated from various soil samples from the St. Norbert College campus and cultured on media containing 1/10 TSY agar. In order to spot potential antibiotic-producing bacteria, 31 different colonies were isolated and patched onto a plate that had a lawn of ESKAPE pathogens *(E.aero, S.epi, E.coli, and E.riff)*. Five of the isolated colonies showed antibiotic production and were then included in a PCR run to amplify the 16S rRNA gene. Future work will include gram staining the other three isolates and biochemical testing to identify metabolic pathways and sequencing of the 16S PCR product.

70 Tiny Earth Project

Laura Vang
Northeast Wisconsin Technical College

For many years, scientists have been continuously researching antibiotics and ways to create new ones. With the human population overusing antibiotics, it has led to resistance among bacterial species and made it more difficult to treat infections with certain antibiotics. One cause of resistance is that the antibiotics regimen are not completed. The Tiny Earth project has given students opportunities to research, test soil samples and look for new microbes in the environment. My hypothesis is that the soil I collected from my parent's farm will contain two antibiotics producing. Since the soil has been exposed to decomposed vegetable matter and fertilizer, it will have microbes that produce antibiotics. During research, I diluted and plated my soil onto a petri dish and isolated the colonies using streak plate technique. I inoculated the microbes on plates with *S. epidermidis* and *Erwinia* bacteria to see if there is any antibiotic activity from my isolate. My plate did not have a double producer, the plate that had antibiotic activity was in the Erwinia plate on the colonies 8 and 9. After the activity was completed, I moved forward to screen the antibiotics against the relatives of ESKAPE pathogens. The result showed that I did not have any potential activity and production against additional ESKAPES, but I did have a one type of colony that was yellow and circular. My hypothesis was correct as there were two antibiotics that were found in the soil from the farm. My research of the antibiotic producing from my parent's farm are still ongoing, therefore some results are to be determined.

71 Antibiotic Producing Bacteria Found in Two Different Soil Types

Haley Calvillo, Alaina Jacobetti
Northeast Wisconsin Technical College

Antibiotics have been around since the 1920's, the first one being penicillin. Antibiotics have become widely used in medicine for treating infections. Recently, bacteria called "superbugs" have been able to resist many types of antibiotics. These drug-resistant infections have caused way too many deaths and the number is only rising. The Tiny Earth organization has included students, instructors, and communities to work towards antibiotic discovery by taking samples from soil. We chose to get our samples from natural soil and soil on a pasture with cattle that have minimal use of antibiotics. Our hypothesis was that the soil that was found within the pasture would have the most antibiotic producing bacteria. We diluted our samples and poured them on a plate to isolate the colonies. The colonies formed were then picked from and tested against *Erwinia* and *S. epidermidis* to see if any of our samples fought against the bacteria. Both the natural soil and the soil found in the pasture had two antibiotic producing bacteria. We each picked one of these bacteria to test against ESKAPE pathogens. Our hypothesis was somewhat correct, the soil that was tested from the pasture and nature soil had produced the same amount of antibiotic producing bacteria. The search for novel antibiotics within pasture soil and natural soil is a current process, therefore results are to be determined.

STUDENT POSTERS

72 Antibiotics from the Wildlife Sanctuary

Paige Kruse
Northeast Wisconsin Technical College

Over the past 30 years the discovery of new antibiotics has decreased at a fairly steady rate. With antibiotic resistance becoming a large issue and not having new antibiotics to try out, this has cause an immense amount of deaths worldwide. Tiny Earth is an organization that is trying to fight back against antibiotic resistance by having students from around the world go out and try to find new ones by analyzing soil. I chose to analyze the soil from the wildlife sanctuary; I thought this would be a very good place to look due to the environment. My hypothesis was that I would find at least 4 antibiotics due to the moist and busy environment my soil was collected from. In class, we diluted the soil in water and used the streak method and were put up against the bacterias *Staphylococcus epidermidis* and *Erwinia* to see if anything in our soil could fight against the bacteria. The soil ended up producing 2 antibiotic microbes, one being a broad- spectrum antibiotic, fighting against both gram-negative and gram-positive bacteria. Then, one bacteria was tested against the ESKAPE pathogens. My results showed 2 antibiotics. My hypothesis was not correct as I had thought there would be 4 but my soil only produced 2. The research with the soil from the wildlife sanctuary is still in process, therefor some of my results will come later.

73 Antibiotic Producers Collected from Heritage Hearing Care Soil

Jonah Zuehls
Northeast Wisconsin Technical College

Humanity has made exponentially tremendous progress in medicine in the last 90 years relative to the three hundred thousand years our species has existed. We have cured so many previously fatal microbial diseases and made the effects of other diseases much less detrimental than previously imagined, but a lot of that progress is at risk of being rendered a thing from the past due to antibiotic resistance. In turn, this means that people will begin dying to diseases previously considered cured. To combat this, the Tiny Earth organization utilizes students to go into the world and collect soil samples in the hopes of finding bacteria capable of producing new antibiotics and neutralizing ESKAPE pathogens, which are highly virulent and resistant to currently discovered antibiotics. I took my soil sample from a tree next to Heritage Hearing Care, which is also my residence. I have owned many animals, such as guinea pigs, rats, mice, and birds. Upon death, I buried each animal in the same spot next to a tree. I hypothesized that I would find at least 5 antibiotic producing microbe species in this location due to the diversity of decomposing animals and health of the tree the soil was next to. I diluted and plated this soil from my sample using the streak plate technique. The microbes found were inoculated and screened against the bacteria *S. epidermidis* and *Erwinia* to see which species produced antibiotics against the bacteria. There was one colony with antibiotic releasing activity against *Erwinia*. This colony was then screened against the ESKAPE pathogens, and activity against *S. epidermidis* was recorded. My hypothesis was incorrect, as there was only one antibiotic producing bacteria species. This research is ongoing; some results are yet to be determined and a conclusion will be available once identification from DNA sequencing is complete.

74 Antibiotic Producing Bacteria Collected from Beaches

Yaritza Cisneros, Melissa Ziebell, Kristin Glowacki, Tara Phillips
Northeast Wisconsin Technical College

The discovery of Antibiotics has transformed human health by being able to treat infections from dangerous pathogens which has subsequently led to the overall reduction in mortality rate that comes from exposure to them. However, the misuse and overuse of antibiotics is the main cause of antibiotic resistant pathogens. We are facing a healthcare crisis due to this misuse and need to find new antibiotic alternatives to better increase our chance of survival as humans in the generations to come.

The Tiny Earth Organization presented us with the opportunity to take samples of soil from our communities and test them in search of the next life-saving antibiotic medicine. Our group has chosen to compare samples taken from different local beaches in Wisconsin that are well traveled by humans. In our own hunt for discovering antibiotic producing bacteria, we collected soil samples from Bay Beach, Wrightstown beach, Ottumba Park Beach, and Selner Beach. Our theory was that our public beaches contain antibiotic producing bacteria because they provide a wide habitat of vital nutrients for microbes to proliferate as well as presenting a unique opportunity of picking up microbes from other animals, such as birds, that travel throughout the states. The beaches are well exposed to water that circulates via a current all along the lakes and rivers touching miscellaneous flora, fauna, animals, insects, and earth. These beaches also have visits from people and their house pets who also bring their own sampling of microbes from their home environments. Due to higher traffic, our hypothesis is that Wrightstown beaches will contain the most antibiotic producing bacteria over that of Bay Beach, Ottumba Beach and Selner Beach. Each group member traveled to their target location and procured a soil sample 2 inches down from the surface and brought it back to the NWTC laboratory for testing. In lab each student performed a soil dilution test, grew, and separated out individual bacterial colonies, picked and tested a suspected bacteria for antibiotic activity against *Staphylococcus* and *Erwinia*, then subsequently tested those same bacteria against the ESKAPE pathogens. The Wrightstown beach soil sample displayed a single colony but showed activity on the *Staphylococcus* and *Bacillus subtilis*. Meanwhile, Bay Beach soil sample only displayed a single colony against *Enterobacter aerogenes*. Bacteria from Ottumba park Beach displayed some antibiotic activity against *Erwinia*, *Staphylococcus* and *Bacillus subtilis*. The bacteria from Selner Beach showed antibiotic activity against *Erwinia* and *Bacillus subtilis*. Our tests are ongoing, so further results are to be determined.

75 The Discovery of Antibacterial Microorganisms at Pamperin Park Creek

Mainor Vang
Northeast Wisconsin Technical College

In modern times, we are facing an antibacterial resistance crisis that can hurt and affect our livelihoods as we use medicine daily. What this means is bacteria in which we have antibacterial medication for can now resist these medicines. The Microbiology class at NWTC has found a way to reverse this by joining the Tiny Earth Organization. Through this organization, students can find antibacterial microorganisms that can go fight against pathogens. First, soil is acquired for the project. I chose soil from a riverbed because I hypothesized that there would be more microorganisms with antibacterial properties due to water. The soil from there was then diluted in the lab and tested against *Erwinia* and *S. epidermidis.* The soil sample contained a double producer which meant it had antibacterial properties against both *Erwinia* and *S. epidermidis.* I can conclude that my hypothesis was correct. More tests are being run on the samples, so more results are pending.

76 Tiny Earth Project

Hannah Dekker, Lizeth Ramos Martinez
Northeast Wisconsin Technical College

Every year we are facing a problem with new bacteria becoming resistant to antibiotics. Which is why we are always having to search for different types of bacteria that will be able to create a new antibiotic. It is time to help discover new antibiotics that is why we decided to participate in the Tiny Earth project. The purpose of this project is to possibly find a bacterium that will have antibiotic producing capability. To achieve this, we went ahead and collected dirt samples from two very distinct places. First, we selected an area that is populated with humans often and the second area we selected was an area where people are less involved. The first place where we collected our soil sample from was from a nearby dumpster located in a dental clinic, the second spot we collected soil from was in a park that is populated more with animals. The environment from both areas contains many differences and factors that affect the soil. We believed that the soil sample from the nearby dumpster had a higher chance of being more populated with bacteria. We diluted our soil and took both samples and made streak plates to produce isolated colonies. After incubating our bacteria, we observed that the soil sample from the dental clinic showed more mold and a bigger variation of bacteria than the soil sample from the park. After running several tests with our bacteria samples, we noticed that when exposed to S.epidermidis , the soil sample from the park showed antibiotic activity. The soil sample from the dental clinic showed no antibiotic activity. Our results showed that our soil samples both have unique characteristic but haven't found any similarities. Our research continues, and we continue to test and learn more about our soil samples.

77 Tiny Earth Project

Leslie Carmona, Marlen Reyes
Northeast Wisconsin Technical College

With the discovery of antibiotics in the early 20th century, bacterial infections all around the world could be cured. With the wide use of antibiotics, strains of resistant bacteria began to continue to grow even with the use of antibiotics. The ultimate cause of this uncontrollable infection has led to death with many individuals who have had bacterial infections.

Due to the antibiotic resistance crisis, the Tiny Earth Project is an experiment used by students all over the nation. The purpose of this experiment is to collect soil samples from unique places to see if any of our samples have new, never before seen antibiotic properties that could be used as future antibiotics to kill off bacterial infections.

In order to figure out if the soil had antibiotic producing bacteria, we used different experiments to help us separate, grow and screen. The methods include but are not limited to soil dilution of the sample obtained, testing the soil in The Master Plate against *ESKAPES, E. coli, S epidermis, B subtilis* to name a few, in total we tested the sample with eight different pathogens.

As we were conducting each experiment we were getting positive reactions and clearance which was very exciting news. The sample showed clearance to 7 spots on the *Erwina cordova* and *Staphylococcus aureus* plates.

Other methods included Colony PCR, this test extracted DNA from our soil sample. Colony PCR is a test to help identify the species of our isolate, in order to isolate and help identify the pathogen we have been working with.

In conclusion the results mean the soil collection, dilution and other tests involved to find our pathogen which has properties of killing bacteria, shows our isolate for further experimenting with possibly finding a new antibiotic to help ease the antibiotic resistance crisis we are experiencing.

78 Researching Antibiotic Activity from Soil Collection

Michaeli Kacsor
Northeast Wisconsin Technical College

As time goes on, bacteria are becoming resistant to more and more antibiotics and that is leading to death around the world. My microbiology class and I have teamed up with The Tiny Earth organization to collect soil samples to perform research for antibiotics. I collected my soil from Klipstine Park in Ashwaubenon because there is a lot of activity at the park. The park is also surrounded by many businesses. My hypothesis was that I was going to find three different microbes that would have antibiotic producing activity. The methods I used were a soil collection where I diluted the soil and then on a plate, I used the streak method to isolate the bacteria. We then tested twenty microbes to see if the soil sample had any antibiotic producers. I then tested them against ESKAPE pathogens for further testing of antibiotic testing. My site had two microbes that had antibiotic activity. My research of the soil is still currently not finished yet, so my results are to be determined.

79 Searching for Antibiotics in the Backyard

Travis Menard
Northeast Wisconsin Technical College

The world is currently facing a problem that very few people outside of the scientific and medical community are even thinking about, and that problem is antibiotic resistant pathogens. In the search for potential new sources of antibiotics, the Tiny Earth project is one of the “tips of the spear” that tasks collegiate learners to examine soil, run and learn about various Microbiological experiments, and potentially identify a new antibiotic that could solve the problem of antibiotic resistance.

My project examines the soil from the garden in my backyard to potentially find a new antibiotic. While I’m somewhat skeptical about the odds of finding anything new in such a common location considering what I have growing there, a few pretty flower arrangements purchased from a big box retailer for instance.

I first gathered a small sample of soil from the brick arrangement and used a soil dilution process to try and obtain a purer sample of any bacteria from it. The lowest dilution used was at a 1/100000 scale. Other experiments then followed, using techniques such as using a “master” plate and testing resistance against *S. epidermidis* and *E. carotovora,* running PCR testing to identify bacteria growing more accurately down to either the genus or species level, and using an “all tester plate” to test production against various bacteria such as *E coli* and *S. epidermidis.*

I was quite surprised to see that in the opening stage of the experiment, the soil dilution followed by streaking on the plates, that I didn’t really have too much bacteria growth. Even running the following experiment that allowed a change of variable, I saw no discernable changes. I assumed that a common spot such as a household garden would’ve had more varied and distinguishable bacterial colonies, but I was wrong.

My results unfortunately didn’t discover any new antibiotics, but it was also sort of what I expected to see going in. I was however surprised to see such a limited amount of varied growth and almost no resistances against any of the bacteria that we tested against. I believe that the hunt for new antibiotics will have to expand a bit farther than someone’s backyard but tests are still ongoing.

STUDENT POSTERS

80 Tested bacterial isolates from Lake Michigan and surrounding soil for their identity and antibiotic producing potential

Abigail Stichter, Chase Gibson, Andrew Jansen
Carthage College

Identification of new antimicrobials is needed due to the rise of antibiotic-resistant pathogenic bacteria. In our search for new antimicrobials this semester, bacteria were isolated from soil and Lake Michigan water samples on the Carthage College campus. Isolation was performed using 10% TSA plates for lake water samples and 50% PDA plates for soil samples. Isolates were tested for antimicrobial activity on ESKAPE relatives of pathogenic bacteria. Four bacterial isolates were selected based on growth factors, colors, textures, and antibiotic production. Initially gram staining and thioglycollate assays were performed to determine the cell shape, cell wall and aerotolerant properties of each bacteria. Further biochemical tests, such as Oxidation-Fermentation tests, Citrate tests, SIM assays, ect. are being performed to characterize each species of bacteria. After the initial hypothesis on bacterial identification based on biochemical test data, a 16S rRNA sequence will be performed on each unknown to confirm its identity.

81 Microbial Activity from Soil in Pulaski

Anastasia Boutris, Miranda Bornemann, Mariah Harris, Kaitlyn Martin
St. Norbert College

Antibiotics have been used since the late 1920s to treat bacterial infections, and shortly after, antibiotic resistance became an issue in healthcare. Thus, there are fewer antibiotics to treat bacterial infections. As more strains of bacteria are becoming resistant to current antibiotics, there is a need to find new antibiotics. Soil is a niche that many bacteria may live in and by diluting a soil sample, the number of bacteria and types can be seen and grown. In collaboration with Tiny Earth Project, bacteria were isolated from a lawn in Pulaski, Wisconsin. 18 distinct colonies were observed and isolated from the diluted soil sample. From the observed 18 colonies, two displayed antimicrobial properties on multiple ESKAPE pathogens. Both bacteria were a cream color and gram-negative rods. Bacteria one was smaller at 10 micrometers while bacteria two was 20 micrometers long. Going forward, the immediate future direction includes PCR and sequencing of the 16 rRNA gene from both isolated bacteria. Additionally, a series of biochemical tests will be performed to further identify the metabolic properties of each bacterium.

82 Isolation and Analysis of Antibiotic Producing Bacteria from Greenhouse Soil

Maddie Thorstad, Natalie Norton, Brianna Tully, Mallory Names
St. Norbert College

To address the growing problem of antibiotic resistance, a greenhouse soil sample was obtained to screen for and isolate potential antibiotic producing bacteria. Both 1/10 TSA and LB agar plates were used to culture the bacteria present in the sample. From that, 10 colonies were patched on the TSA agar plates, and 12 colonies were patched onto the LB agar plates. In order to test for antibiotic production, these 22 isolated colonies were grown on TSA and LB plates containing lawns of *Staphylococcus epidermidis* and *Escherichia coli.* Four colonies were selected as possible antibiotic producing colonies to be further investigated. A PCR was run to amplify the 16s rRNA genes of the selected colonies. Gel electrophoresis was performed to confirm successful amplification. PCR products were sent to undergo genome sequencing.

83 Dirt for Defense

Ashley Cook
Northeast Wisconsin Technical College

Misuse of antibiotics over the last century has created a quickly overwhelming resistance from bacterial pathogens in medicine. The goal was to collect a soil sample; identify individual colonies; extract and isolate a single colony and test for antibiotic production against well-known pathogens. Sample was obtained in Howard, WI from the banks of Duck Creek. The diverse environment from flooding and drying throughout the year makes this area stand out for possibility of undiscovered antimicrobial producers. Promising results were found from two isolated colonies when exposed to *S. epidermidis* and *E. caratovora* Upon further testing the stronger of the two previously mentioned colonies was then exposed to ESKAPE pathogen relatives with visualized hinderance of growth in five of eight pathogens. Completion of PCR and gel electrophoresis showed affirmation that the DNA from this microbe was obtained. Continued testing will be completed in hopes of identifying an undiscovered antimicrobial producing microorganism.

STUDENT POSTERS

84 Antibiotics in a Soil Sample

Maxwell Leonhard, Matthew Trotter, Gabe Smith
St. Norbert College

Due to the constant increase in antibiotic resistance, the task of discovering new antibiotics is essential to combat resistance in bacterial infections. In collaboration with the Tiny Earth Project, a soil sample yielded five distinct types of bacteria colonies that were cultured in 1/10 TSY agar media. The soil was acquired from the St. Norbert College campus in the vicinity of a garbage collection area. To detect antibiotic-producing bacteria, the five bacteria types were plated on a lawn of an ESKAPE pathogen. Three of the five colony types showed signs of antibiotic production. One was visually emitting a brown-colored secretion, while the other two caused open areas on the bacteria lawn along their border. The colonies were isolated, the 16S rRNA gene was sequenced, and sent off for sequencing. The DNA of the blue bacteria colony was then purified from the PCR reaction of the blue bacteria colony. Additional research will follow based on the sequencing results.

85 Isolation of potential antibiotic producing soil bacteria

Jared Brown, Giovanni Basanese, Callie Orlando, Jillian Tringali
St. Norbert College

Due to the intrinsic ability of disease-causing bacteria to form antibiotic resistance, it has become pertinent to our studies of microbiology to determine specific organisms that produce novel antimicrobial agents. Thus, bacteria were isolated from soil samples from the Niagara Escarpment region of Northeastern Wisconsin due to the large concentration of a myriad of species there. To identify antibiotic producers, five colonies were isolated from a nutrient agar patch plate of 11 potential colonies. The colonies exhibited the following characteristics and were labeled as: gram-positive, filamentous, cocci (M); gram-negative, bundled, cocci (B); gram-positive cocci (2); gram-negative rods (3); and gram-negative rods (5). To identify the samples, a series of biochemical tests will be performed to determine metabolic activity. PCR amplification of the 16S rRNA gene has been conducted and the PCR products will be purified and sequenced to confirm correct speciation.

86 The Isolation of Antibiotic-Producing Bacteria from House Plant Soil

Jessica Magalski, Joseph Fischer, Grace Markovitz, Andrew Eikman
St. Norbert College

Antibiotic resistance of pathogenic bacteria has increased as a consequence of antibiotic overuse. In contribution to the Tiny earth project, this experiment isolated groups of bacterial colonies from two soil samples acquired from indoor houseplants (39 x 10^5 cfu/mL) (33 x 10^5 cfu/mL) to test for antibacterial activity. The techniques included serial dilution of the soil sample, patching of the colonies, antibiotic sensitivity plate testing, and PCR to test for the presence of the 16S ribosomal RNA genes. The bacterial colonies that exhibited antibiotic resistance when plated on the four bacterial solutions individually, were selected to conduct a PCR. The ESKAPE pathogen bacterial solutions included *Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis,* and *Pseudomonas putida*. Bacteria 14 from group one exhibited antibacterial properties when plated on *Bacillus subtilis* and when stained exhibited gram-positive rod morphology. Bacteria 14 from group two exhibited antibacterial properties when plated on *Bacillus subtilis* as well, and exhibited gram-positive rod morphology. Finally, Gel electrophoresis of the PCR product confirmed the existence of the 16S Ribosomal RNA, and purification for a second PCR was performed in order to obtain sequence information from the lab. The bacterial genomes will be analyzed further to identify genes that may contribute to antibiotic production.

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