3RD Annual

Small World Initiative (SWI) Symposium

Small World Initiative
crowdsourcing antibiotic discovery

asm microbe 2016

June 16-20, 2016
Boston, MA

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Small World Initiative

crowdsourcing antibiotic discovery

Third Annual
Small World Initiative Symposium

at

American Society for Microbiology

(ASM Microbe 2016)

June 16-19, 2016

Boston Convention and Exhibition Center

Boston, MA

Symposium Schedule

Thursday, June 16, 2016

Arrival and registration	7:00 AM-8:00 PM
ASM Microbe Opening Keynote (Bill Gates)	5:00 PM-6:00 PM
Opening Reception	6:30 PM-8:00 PM
SWI Student Scavenger Hunt Virtual Launch	3:00 PM
SWI Student and Faculty Meet and Greet	7:00 PM

Friday, June 17, 2016

ASM General Session Distinguished Lectures	5:45 PM-7:30 PM
SWI Poster Set-up	7:30 PM
SWI Poster Session	
A. SWI student & faculty posters 1-10	8:00 PM-8:40 PM
B. SWI student & faculty posters 11-20	8:45 PM-9:25 PM

Saturday, June 18, 2016

ASM General Session President's Forum	11:00 AM-12:30 PM
SWI Keynote Address: *A Conversation with Susan Whoriskey*	6:00 PM-6:45 PM
SWI Symposium Awards	6:45 PM-7:00 PM
SWI Student & Faculty Group Photograph	7:00 PM
SWI Faculty Tapas Session	7:15 PM-8:15 PM

Sunday, June 19, 2016

ASM General Session: Antimicrobial Research Award Lecture	11 AM-12 noon

*All SWI activities will be held at the Seaport World Trade Center, Commonwealth Complex

Small World Initiative
crowdsourcing antibiotic discovery

SWI Leadership

From right to left

Erika Kurt, SWI President and CEO

Jo Handelsman, SWI Founder

Nichole Broderick, SWI Partner Instructor Lead

Symposium Organizing Committee

Jean Schmidt
University of Pittsburgh

Mustafa Morsy
The University of West Alabama

Betsy Roberts
Southern Connecticut State University

Eric Warrick
State College of Florida

About the Small World Initiative

Formulated at Yale University in 2012 by the current Associate Director of Science at the White House, Jo Handelsman, the Small World Initiative™ (SWI) is an innovative program that encourages students to pursue careers in science while addressing a real-world health threat – the diminishing supply of effective antibiotics. SWI centers around an introductory biology course in which students perform hands-on field and laboratory research on soil samples in the hunt for new antibiotics. Through a series of student-driven experiments, students collect soil samples, isolate diverse bacteria, test their bacteria against clinically-relevant microorganisms, and characterize those showing inhibitory activity. This is particularly relevant as over two thirds of antibiotics come from soil bacteria or fungi.

SWI's novel approach harnesses the power of active learning to achieve both educational and scientific goals and provides a unique and sustainable platform to replenish the antibiotic pipeline by identifying suitable candidates for testing. Currently, SWI's course is in 108 schools (98 colleges) across 33 US states, Puerto Rico, and ten additional countries – Belize, Canada, Iraq, Ireland, Jordan, Malaysia, Nigeria, the Philippines, and the UK, and has impacted more than 8,000 students.

Please visit www.smallworldinitiative.org to learn how to get involved or support us.

Twitter: @Team_SWI

Message from SWI's President and Chief Executive Officer

Dear SWI Community,

This has been an exciting year for the Small World Initiative™ (SWI), and while a lot of work remains, a number of key milestones have been reached! I want to extend my sincere thanks to SWI's Partner Lead – Nichole Broderick – and our Committee Leaders – Ana Maria Barral, Brittany Gasper, Debra Davis, Elia Crisucci, Jean Schmidt, Kristen Butela, Mustafa Morsy, Paula Soneral, Samantha Gruenheid, and Todd Kelson – for all of their hard work over the course of the year, which is the backbone of this collaborative initiative.

Key Milestones Reached

New Possibilities – In March 2016, SWI became its own nonprofit organization with its board focusing exclusively on this quickly expanding program.

Fast Growth – Since its inception in 2012, SWI has grown rapidly:

- 2013-2014, 30 Colleges in the US
- 2014-2015, 60 Colleges in 5 Countries
- 2015-2016, 98 Colleges in 9 Countries, Pilot High School Program
- To date, we have impacted more than 8,000 students and 140 instructors.
- 2017 Projection: Over the next 12 months, SWI will expand to more than 150 undergraduate and high school institutions and will be adding at least two new countries – Spain and India. The majority of instructors from these new institutions will be trained at the University of Connecticut this month.

Recognition – Hundreds of our students and partner instructors have presented their original research at major conferences around the world and even on Capitol Hill. Just last month, Nichole Broderick's SWI talk at the White House was live streamed for the launch of the National Microbiome Initiative!

Published Results – In March 2016, the Journal of Microbiology & Biology Education (JMBE) published an article supporting our educational impact. The authors, including the late Dr. Joe Caruso, found that our program improved students' lecture grades and California critical thinking skills test scores.

Opportunities – This year, we launched our online portal for students and faculty to locate relevant opportunities. We also established four awards for students and faculty that align with SWI's pedagogical and scientific goals, distributed three travel grants, and hosted a social media contest to encourage online student engagement.

On the Horizon

Material Development – We will finalize the new biosafety materials and our 4th edition of course materials over summer 2016.

Central Repository – We are collaborating with the University of Connecticut and Yale University to establish a central repository to store soil samples. This is a critical first step to establishing the screening laboratory.

I am looking forward to continuing on this journey using innovative strategies to inspire the next generation of scientists, increase scientific literacy, and confront the antibiotic crisis head on.

All my best,

Erika Kurt

President & CEO

Keynote Speaker

The Keynote Speaker for the 2016 Small World Initiative (SWI) Symposium is Dr. Susan Whoriskey. On Saturday, June 18th at 6pm, Erika Kurt, President and CEO of the Small World Initiative, will sit down with biotechnology entrepreneur Susan Whoriskey, Ph.D., a founding member of Momenta and Cubist Pharmaceuticals. A leader in the biopharmaceutical industry, Dr. Whoriskey was instrumental in bringing to market Cubicin (daptomycin), a lipopeptide antibiotic active against drug-resistant bacteria, in 2003. Dr. Whoriskey earned her Ph.D. in Molecular Biology from the Molecular Biology Institute-University of California, Los Angeles and a Bachelor of Science in Microbiology from the University of Massachusetts-Amherst. Dr. Whoriskey will share some tales from the lab bench, from the boardroom, and from her inspirational scientific career.

Table of Contents

Abstracts

1. 10% Trypticase Soy Agar and R2A Medium Better Support Culture of Diverse Antibiotic-Producing Soil Bacteria than Luria Broth Agar

Ashwinee Manivannan, Jacob McKenzie, Luke Pomrenke, and Jean Schmidt

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA

Antibiotic resistance is a grave problem worldwide due to the overuse of antibiotics. Our research as part of the Small World Initiative has focused on finding new antibiotics in soil bacteria since more than two-thirds of antibiotics originated from soil microbes. We collected soil samples from around the University of Pittsburgh campus, determined the physical and chemical characteristics of each sample, cultured the soil on various media, and tested the isolated bacteria for antibiotic production against relatives of clinical pathogens. Antibiotic producers were characterized by Gram staining, and identified either with PCR amplification and sequencing of 16S rDNA, or via BioLog analysis. Organic extract of cell metabolites was tested to confirm antibiotic activity. Of the growth media tested, 10% trypticase soy agar (TSA) and R2A medium supported the greatest diversity of bacteria, each scoring a maximum of 6.0 on the Exponential Shannon Index. Isolate AM-TSA10-14 is rod-shaped and inhibited the growth of *Escherichia coli*. Colony PCR was not successful, however BioLog analysis determined that the isolate is of the *Pseudomonas* genus. Organic extract of this isolate also inhibited *Escherichia coli*. Isolate JM-TSA10-1 is rod-shaped and produced antibiotics against *Staphylococcus cohnii*. 16S rDNA sequencing revealed its genus to be *Bacillus*. Isolate JM-TSA10-3 is filamentous and Gram positive, and inhibited *Staphylococcus cohnii*. 16S rDNA sequencing revealed the isolate to belong to the genus *Streptomyces.* 10 % TSA and R2A are promising media for isolation of diverse soil bacteria, increasing the chances of finding a novel antibiotic to combat the antibiotic resistance crisis.

2. The Big Dirty: Unearthing Antibiotic-Producing Bacteria from Bourbon Street in New Orleans, LA

Megan Moore, Mary Miller, and **Ajay Prasad**

Science, Technology, Engineering and Mathematics Department, Baton Rouge Community College, Baton Rouge, LA

Through a partnership with the Small World Initiative formulated by Yale University in 2012, we sought to isolate antibiotic producing bacteria in order to address the worldwide concern of unavailability of effective antibiotics. This poster will present the finding from bacterial isolates obtained from a soil sample near a sewer grate off of Bourbon Street in New Orleans, Louisiana. A serial dilution was performed and 21 bacterial isolates were selected. The isolates were screened for antibiotic production against ESKAPE pathogens and morphological and metabolic analysis was conducted on selected isolates. DNA was isolated and the 16S gene was sequenced at Pennington Biomedical Research Center. The sequence results were used to further identify the isolates and construct a phylogenetic tree to determine the relationship between the organisms obtained using traditional isolation techniques.

3. The Isolation and Characterization of Marine Antibiotic Producing Bacteria from Sarasota Bay, Florida

Dominick Christou-Ader, and **Eric Warrick**

Natural Science Department, State College of Florida, Bradenton, FL

The current emergence of a plethora of multi-drug resistance bacteria has left us with a dwindling variety of effective antibiotics to fight infections. The once elementary infection may now be of great concern as the cause of the infection may have developed the ability to combat one of our only outside defenses, antibiotics. This emerging crisis has stimulated the need for researchers to discover and develop new antibiotics to target these multi-drug resistance bacteria. In order to discover new antibiotics, samples should be taken from more unique environmental locations. To accomplish this, sediment and water samples taken from offshore and inshore sites along the Gulf of Mexico and Sarasota bay were collected and cultured on various media looking for antibiotic producing bacteria. Media recipes included potato dextrose agar, lysogeny broth, and actinomycetes isolation agar. All of the media were supplemented with 0.5M NaCl and 100µg/ml cycloheximide. Incubation conditions were designed to be optimal for the enrichment and isolation for marine Actinomycetes. All samples were plated at dilutions yielding plates that contained 30-300 colonies and then transferred to a gridded master plate for individual testing. Each colony was tested against several ESKAPE safe relatives; *Staphylococcus epidermidis*, *Bacillus subtilis, Enterobacter aerogenes*, and *Citrobacter freundii* as well as the non-pathogenic marine organism *Chromohalobacter salexigens* for the ability to produce a zone of inhibition. Colonies that showed inhibition of an ESKAPE safe relative were preliminarily identified using *16s rDNA* gene. Initial testing resulted in *Streptomyces aurues*, *Bacillus amyloliqefaciens*, *Chromobacterium aquaticum* and an unknown species of *Microbacterium.*

4. A red *Vibrio* species' potential as a novel antibiotic.

Kristin Burnham, Emily McClure, Patricia Rossi, and Nichole A. Broderick

Department of Molecular & Cell Biology, University of Connecticut, Storrs, CT

Soils samples from the rhizosphere were taken from the UConn campus in Storrs, CT and a salt marsh in Norwalk, CT. Forty-one bacterial colonies were isolated from the samples and were tested for antimicrobial activity against safe relatives of the ESKAPE pathogens. An isolated red *Vibrio* species had especially strong inhibitory effects towards a broad range of pathogens. Extract from the *Vibrio* sp. did not exhibit eukaryotic activity, suggesting it may have potential as a novel antibiotic producer. Based on 16S rRNA sequencing the active Vibrio sp. was most closely related to *Vibrio rhizosphaerae* or *V. rubra.*

5. Isolation and identification of antibiotic producing bacteria from soil

Marne Bailey, Jeannette Pifer, and **Elisabeth Hardin**

Biology Department, Lewis University, Romeoville, IL

Most antibiotics currently prescribed to treat infections are derivatives of natural compounds produced by bacteria and fungi. Unfortunately, most antibiotics will not remain clinically effective long-term due to the increasing frequency of antibiotic resistance. Increasing instances of multidrug resistance coupled with a decrease in the discovery and development of novel antibiotics is setting the stage for a global healthcare crisis. To combat this increasing public health threat, the idea of "crowdsourcing" to isolate and identify new antibiotic producers and new drugs is growing in prominence. This work represents the results of implementation, in our undergraduate microbiology laboratory, of the Small World Initiative crowdsourcing for antimicrobial discovery.

Suspensions of soil samples from thirty-two locations were plated to yield individual colonies. Diverse colonies and any colonies exhibiting clear zones of growth inhibition of neighboring cell growth were selected. Isolates were tested for antimicrobial activity against a panel of ESKAPE pathogen safe relatives. Isolates exhibiting antimicrobial greatest antimicrobial activity against the ESKAPE panel were identified using 16S rRNA sequencing followed by nucleotide BLAST sequence alignment. Additionally, isolates exhibiting the most significant antimicrobial activity against the ESKAPE tester strains were subjected to organic extraction with ethyl acetate. The organic extracts were dried, resuspended and spotted onto LB agar plates inoculated with the same ESKAPE strains to test for organic antimicrobial activity.

Over 400 diverse colonies were tested against a panel of Gram positive and Gram negative ESKAPE pathogen safe relative strains. Sixteen examples that exhibited the most substantial growth inhibition of tester panel strains were sequenced and subjected to the organic extraction process. Sequencing identified species of the genera *Bacillus, Pseudomonas, Microbacterium, Streptococcus, Staphylococcus, Streptomyces* and *Enterobacter*. One particular isolate, ELHA18b, identified as a strain of *Bacillus pumilus,* was found to actively grow and produce an active organic antimicrobial across a range of temperatures from -80C to 42C.

The data support the concept that soil yields a diverse population of bacteria that produce active antimicrobial compounds and may harbor as yet unidentified antibiotic compounds.

6. Purification and Characterization of a Novel Antibiotic Molecule from a species of *Streptomyces*

Matthew Greenwald, Rachel Glasser, and Lingfeng Liu

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA

The overuse of antibiotics in modern culture has created a global pandemic of antibiotic resistant bacteria, which are currently gaining prevalence in hospital infections. To combat this pandemic, we returned to the study of soil bacteria, from which two thirds of the antibiotics used today have been isolated. Our general strategy for discovering antibiotics began with the creation of an initial culture of a confirmed antibiotic-producing bacterium. The molecules produced were extracted and separated though the use of various chromatographic techniques. Once the molecules were individually isolated, they were characterized using spectroscopy methods to identify the structure of the antibiotic molecules being produced. We worked with a bacterium isolated from soil at the University of Pittsburgh, which was analyzed for antibiotic production, sequenced, and found to be from the *Streptomyces* genus. After isolating and purifying the antibiotic molecules produced, we began characterizing one of the molecules in further detail. Running high-resolution mass spectrometry gave us a molecular mass of 628.32. Then we performed hydrogen and carbon NMR to gather information about the chemical environment of the atoms. We were able to determine various substituents on the molecule such as 3 para-substituted aromatic rings, a cis and trans-alkene, and a benzene with an isopropyl group. Through this information, we generated a hypothesized base structure, which we could not find in any databases or papers. Therefore, it could potentially be a novel antibiotic. Further structural analysis via 2D NMR will be performed to elucidate the structure of the molecule.

7. Uncovering Tomorrow's Medicine: The Search for Novel Antibiotics in Dirt

Haley Turner, Abigail Coley, and Mustafa Morsy

The University of West Alabama, Livingston, AL

The goals of the Small World Initiative (SWI) are to engage students in authentic research experience and to crowd source discovery of novel antibiotic. Due to increasing number of drug-resistant bacteria**,** there is a dire need for discovery of novel antibiotics. At the University of West Alabama, we tested various soil samples from east Mississippi and west Alabama in search for antibiotic producing bacteria. Unknown bacteria were isolated from soil samples using sterile water, plated on LB agar media and grown at 34^{o}C for 16 hours. The abundance of soil bacteria varied significantly ranging from 31 to > 1,000 CFU with 5 to 17 different phenotypes based on the soil sample. Each student tested 95 unknown bacterial colonies for antibiotic production by co-plating with *Escherichia coli* and *Acinetobacter baylyi*. Screening of 285 unknown bacteria yielded 30 inhibition zones, indicating antibiotic production from these unknown bacteria. Furthermore, the 30 identified antibiotic producing bacteria were screened against four additional ESKAPE pathogens to test the antibiotic efficacy. Some of the unknown antibiotics were effective against some pathogens and not for others. Identification of antibiotic producing bacterial is in progress using morphological characteristics, biochemical testing, and Polymerase Chain Reaction of the 16S r-RNA followed by Sanger sequencing**.** These results indicate a specificity of the antibiotics produced. Our research findings could potentially help save numerous lives lost to antibiotic resistant bacteria. Additionally, novel antibiotics can save about $35 billion due to care of patients infected with antibiotic resistant bacteria.

8. Small World Initiative at McGill University: Crowd-sourcing Antibiotic Discovery in the Fight Against Antimicrobial Resistance

[1]Connie Shen, [1]Tyler Cannon, [1]Emma Hignett, [2]Jean-Guillaume Edmond Rheault, [2]Julie Jeukens [2]Luca Freschi, [2]Roger C. Levesque and [1]Samantha Gruenheid.

[1]Department of Microbiology and Immunology, McGill University, Montreal, Canada

[2]Institut de Biologie Intergative et des Systemes (IBIS), Universite Laval, Quebec City, Canada

The Small World Initiative is a multi-institutional, collaborative effort to tackle the looming antibiotic resistance crisis by crowd-sourcing the process of antibiotic discovery, whilst providing undergraduate students an opportunity to partake in authentic scientific research. Students all around the globe isolate and culture soil microbes to be screened for antibiotic activity against safe ESKAPE relatives. At McGill University, the first Canadian university to implement a SWI program, 229 students taking the MIMM212 Introductory Microbiology Lab course in the Fall of 2014 and 2015 cultured over 5000 isolates from soil collected in urban and suburban Montreal. Of those cultured isolates, over 350 were identified to have antibiotic activity, and over 200 isolates were chosen for further characterization by biochemical, microbiological, observational, and molecular methods. The antibiotic screens revealed that the isolates most commonly produced antibiotics against *B. subtilis* and least frequently against *E. raffinosus*. Additionally, 16s rRNA sequencing and subsequent BLAST analysis showed isolates originating from several distinct phyla. During the summer of 2016, chemical extractions were done for selected isolates. The extractions demonstrated that the antibiotic activity is retained when extracted and thus have compounds in solution. Finally, to determine whether or not the discovered antibiotics are indeed novel, genome sequencing and extraction analysis will be done later this summer.

9. Discovery of a Novel Prodigiosin Producing *Vibrio species* from the Gulf of Mexico

Stephanie Morgan[1], Eric Warrick[2], Brittany Gasper[3], and **Katherine Walstrom[1]**

[1]New College of Florida, [2]State College of Florida, and [3]Florida Southern College

As a part of the Small World Initiative project, environmental samples taken from a salt flat in Bradenton, FL led to the discovery of a *Vibrio* organism capable of producing a bright pink compound. A crude extract of cultured bacterial cells contained a broad spectrum antibiotic capable of inhibiting growth of several ESKAPE safe relatives. As a continuation of the project, full genomic sequencing of the isolate and analysis of the genomic sequence utilizing the genome browser Artemis, NCBI's BLAST and Clustal Omega was performed. Identification of the isolate was done using a polyphasic approach including biochemical characterization and multi-locus sequence analysis with genes found in the isolate genome. The isolate was identified as a member of the *Vibrio* genus within the *gazogenes* clade.

Furthermore, the pigment compound was isolated using a multi-step purification procedure and analyzed with UV-Visible spectroscopy and tandem mass spectrometry. Prodigiosin was identified as being the main component of the extract and two other simple derivative prodiginine pigments were identified based on the known molecular weight and fragmentation patterns identified in the mass spectra. Additionally, a prodiginine biosynthesis gene cluster consisting of 13 genes with sequence homology to prosigiosin biosynthesis gene clusters in *Serratia* species was found in the genome of the isolate. Prodigiosins are biomolecules produced by a variety of bacterial organisms as secondary metabolites. They are often brightly colored pigment molecules with unique chemical properties that have been studied for their applications in both medicine and industry.

10. Bacterial Isolates from Schenely Park Show Antibiotic Activity Against the Tester Strains *Staphylococcus cohnii* and *Staphylococcus epidermidis*

Nicole Hunzeker, Katie Grobengieser, Rishab Shetty, and Elia Crisucci

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA

Microbial communities in soil are generally high in abundance and diversity, making it an excellent source of antibiotic-producing bacteria. Because antibiotics are misused and overprescribed, harmful pathogens are rapidly evolving drug resistance, creating a pressing demand for new antibiotics. We aimed to determine which soil conditions are rich with antibiotic-producing bacteria and to isolate novel antibiotic-producing bacteria. Soil samples were collected from Schenley Park near the University of Pittsburgh campus and serially diluted to culture individual bacterial colonies. The soil samples contained an abundance of bacteria with the highest values of 6.10×10^8 CFU/g on LBA media and 2.27×10^7 CFU/g on TSA media. Colonies that showed interesting morphological features were selected and tested for antibiotic activity against safe relatives of clinically relevant human pathogens. The frequency of antibiotic producing bacteria was highest on LBA media with 23.1% and 11.11% of isolates showing antibiotic production against at least one tester strain. Three antibiotic-producing isolates were selected for further study. We performed Gram staining to characterize each isolate and determined the genus of each isolate through 16S rDNA sequencing. NH-LBA-25 inhibited *Staphylococcus cohnii* and *Staphylococcus epidermidis* and is a Gram-negative, rod-shaped bacterium in the *Pseudomonas* genus. NH-LBA-18 inhibited *Staphylococcus epidermidis* and is a Gram-variable bacterium in the *Bacillus* genus. RS-TSA-6 inhibited *Staphylococcus cohnii* and is a Gram-positive, spherical bacterium in the *Bacillus* genus. In the future, further steps will be taken to purify and determine the structure of the antibiotic compound produced by isolate NH-LBA-25.

11. Classifying Coastal Bacterial Colonies Isolated from Sarasota Bay, Florida with Antimicrobial Activity

Michael Grandalski, and **Eric Warrick**

Natural Science Department, State College of Florida, Bradenton, FL

Overuse of antibiotics has become a serious threat to medicine as many pathogens have become resistant to commonly used antibiotics. The aim of the Small World Initiative is to use our environment to find bacteria that can naturally fight off pathogens and identify them for possible use in medicine. Bacteria were isolated from Sarasota Bay and tested for antimicrobial activity against the ESKAPE-safe bacterial strains. The samples that produced an antimicrobial response to the ESKAPE-safe bacterial strains were then classified by DNA sequencing the 16s *rDNA* gene, *gapA, rpoO,* and *rpoB*. An electrophoresis gel was performed to ensure amplification of the targeted genes was successful during PCR. The amplified DNA samples were sequenced at Yale University and examined through the National Center for Biotechnology Information (NCBI) database for identification. Based on the sequencing results, the unknown bacteria were identified as *Rhodococcus sp., Bacillus sp*., *Paenibacillus sp*., and *Bacillus pumilus*. The identified samples were then subjected to chemical extraction using ethyl acetate as the organic solvent. The chemically extracts were then tested against the previously mentioned ESKAPE-safe bacterial strains to determine if the antimicrobial compound(s) were successfully extracted. The samples identified were shown to produce antimicrobial activity against *Escherichia coli, Staphlococcus epidermis, and Enterobacter aerogenes*.

12. Incidence of antibiotic producing bacteria in grassland and hemlock forest soils support competition-sensing based models of interference competition

Leen Ajlouni, Abigail Bergman, Alexandra Hill, Siphokazi Kargbo, Autumn Mineo, and **Jan A.C. Vriezen**

Department of Biological Sciences, Smith College, Northampton, MA

Due to the saturation of sampling soils for antibiotic producers it is increasingly hard to find novel antibiotic producing bacteria. Therefor, a hypothesis driven approach aimed to understand the *in-situ* condition leading to antibiotic production will allow directed searches for novel antibiotics. Due to the Competitor Sensing Interference Competition based nature of antibiotics producers, our first hypothesis is that the incidence of antibiotic producing bacteria positively correlates with bacterial load. Secondly, because environments with a low bacterial load tend to be higher in diversity, we predict more novel antibiotic producers to reside in such an environment.

Therefor, the bacterial load of four different soil samples was determined and colonies were screened for antibiotic producing abilities on indicator lawns of *S. epidermidis* and *E. coli* followed by 16S sequencing.

The bacterial load expressed in CFU's/g dry soil decreased from $4.33+/-0.72x10^6$ in Grassland surface soil, $2.90+/-0.41x10^6$ at 20 cm depth, $1.63+/-0.32x10^6$ in the hemlock forest A-horizon, and $4.39+/-0.84x10^5$ in the hemlock forest B/C-horizon. Of the colonies emerging on the plates, 21.1, 16.5, 13 and 13.1 percent could inhibit the indicator lawns, which positively correlates with the bacterial load of the sample (R^2=0.96) and strongly supports our first hypothesis. This positive correlation is caused by activity on *S. epidermidis* (R^2=0.99), but is negative on *E. coli* (R^2=0.5). Support for our second hypothesis is weak, since the diversity of antibiotic producers increases with a lower bacterial load in Forest soil while the opposite was observed in Grassland soil.

13. A Multidisciplinary Approach to Discover Antimicrobial Molecules

Jayshree D. Patel, **Juliann L. Downing**, and Huda Makhluf

Mathematics and Natural Sciences, National University, San Diego, CA

The goal of the "Small World Initiative" is to discover new antibiotics from soil bacteria. A study was carried out to obtain potential strains of bacteria from an urban worm farm soil sample. Bacterial colonies were isolated by first diluting the soil in broth media then applying the crowded plate isolation technique. Standard protocols were employed to isolate distinct types of bacterial strains. Antimicrobial testing was conducted using the Kirby-Bauer method. Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* were selected to study antimicrobial efficacy. The potent isolates were characterized by16sRNA sequencing using 27F and 14R universal primers. The colony dubbed JPT8 had the largest zone of inhibition against *S. aureus,* so it was selected for antimicrobial molecule extraction. Active biomolecules were obtained by using large scale fermentation, followed by organic solvent extraction. Results from the validated crude extract confirmed the presence of active antimicrobial agents. Thin layer chromatography (TLC) was employed to determine which fraction was producing the antimicrobial reaction. The 16sRNA sequence did not have an exact alignment in NCBI's database that suggests the JPT8's classification. The potential uniqueness of this isolate may lead to the discovery of a novel compound in an era of increased antibiotic-resistant threats.

14. Investigation of an Antibiotic Biosurfactant Produced by a Plant-Growth Promoting Strain of *Pseudomonas koreensis* Isolated from Connecticut

Laeticia Iboki, Jacqueline Thurber, and Elizabeth Lewis Roberts

Biology Department, Southern Connecticut State University, New Haven, CT

The 16s rRNA sequence of a fluorescent bacterium isolated from the rhizosphere of a Japanese Maple tree (*Acer japonica*) in Connecticut shared 97% identity with *Pseudomonas koreensis*. Evolutionary history of the Connecticut isolate was inferred by Maximum Parsimony analysis of the 16s sequence compared to other Pseudomonas strains including three *Pseudomonas koreensis* strains isolated in Mexico and two strains from Korea. The Connecticut isolate clustered with *P. koreensis* strains from Korea (95% bootstrap support). An atomized oil droplet assay detected a putative CLP biosurfactant produced by the new *P. koreensis* isolate grown on tryptic soy agar (TSA). A total of 0.3 µg (in 30µl methanol) of the biosurfactant spotted onto TSA plates showed marked antibiotic activity identified through clear zones of inhibition against *Staphylococcus epidermidis*, and *Bacillus subtillis* overlaid on the plates. Addition of the bacterium to soils of twenty individual heirloom tomato (*Solanum lycopersicum*) plants resulted in significantly increased biomass (38% increase) over control plants. Furthermore, only 4 out of 48 individual tomato plants inoculated with the bacterium exhibited leaf rolling after being subjected to 3 days with no water at 40 °C, in contrast to 100% leaf rolling observed on un-inoculated controls.

The ability to produce a biosurfactant differentiates our strain from the Korean isolates. In addition, our findings indicate that the Connecticut *P. koreensis* isolate could be used as a biological control agent, or to improve the health of tomato plants exposed to abiotic stresses.

15. The Small World Initiative: Empowering Latinas into Computational Biology

Lyanne Casillas-Santiago, Luis Valentín-Alvarado, Lilliam Casillas-Martínez and Esther Vega-Bermúdez.

University of Puerto Rico-Humacao, Humacao, PR

Studies by the National Science Foundation indicate that women's participation in Computational Sciences (CS) is less than 18%. Among women of color, including Latinas, participation decreases to 4.9%. A main problem alluring Latinas to CS is the lack of femininity in these male-dominated careers. Consequently, a Teaching to Increase Diversity and Equity in Science (TIDES) initiative to increase the number of Latinas entering into CS was implemented. The empowering program "*Cybernetic Girls can be Pinky*" allure Latinas into Computational Biology. The program consist of revamping three Biology core courses with more quantitative analysis experiments, the offering of R and Phyton programming workshops to Biology students complemented with gender inequality conferences. To revamp the Molecular and Cell Biology Laboratory we implemented the Small World Initiative (SWI). All SWI modules were translated to Spanish and students identified more than 25 unique isolates with antimicrobial activities. Students also participated in oral reports, class discussions and final poster preparations. Comparison of the pre and post-tests that measures critical-thinking skills in the lab indicated Hake's Gain (up to 66% differences). Overall evaluation of the programming workshops was 4.3 (with 5 as excellent), students only faced problems making graphs using ggplot2 and in how to simulate different distributions in a study. To fully empower undergraduate students we also provided a Women in Science Course that emphasize the accomplishments of Latinas in Science nowadays. We plan to incorporate the changes, surveys and programming and gender workshops as part of the undergraduate Biology program at our institution.

16. Taking the next step: Antibiotic Compound Isolation from *Lysobacter antibioticus*

Coleman Pinkerton, and Lingfeng Liu

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA

Our healthcare infrastructure is currently plagued by antibiotic resistant pathogens, an unaddressed threat that continues to grow in frequency and severity. The Small World Initiative, an undergraduate biology lab course taught at many universities globally, crowd-sources the search for novel antibiotic producing microbes from local soil populations. Building on the success and the model of the Small World Initiative, this pilot organic chemistry lab course seeks to isolate and characterize the antibiotic compounds produced by microbes in the Small World Initiative. We repeated experiments in the isolation of antibiotic compounds from a strain of *Lysobacter antibioticus*. We used liquid-liquid separation in ethyl acetate to produce a crude extract, with which we preformed HP20, then silica gel, and finally HPLC chromatography to isolate a variety of distinct compounds. Following each isolation step, we sampled extracts in an activity assay against lawns *Bacillus subtilus* on LBA, with positive results confirmed by a zone of inhibition in which *B. subtilus* did not grow. We used NMR, IR, and UV spectroscopy and LC Mass Spectrometry data to analyze the structure of several isolated compounds with confirmed antibiotic activity. We succeeded in isolating two similar antibiotic compounds from a strain of *Lysobacter antibioticus*. We ultimately used these techniques to isolate antibiotic compounds from unidentified microbes isolated in SWI courses in the second half of this course. With greater availability, this organic chemistry lab curriculum could expand the crowd-sourced search for antibiotics into the isolation of antibiotic compounds.

17. Bacteria from University of Pittsburgh Soil Produce Antibiotics Against Relatives of Gram-Positive and Gram-Negative Pathogens

Vincent Piro, **Stephen Reber**, Olivia Wiesner, and Jean Schmidt

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA

Bacterial pathogens have acquired increasing resistance to many antibiotics and have depleted an already limited number of effective antibiotics. As more antibiotics become less effective, countless patients' lives are at risk due to increasingly dangerous pathogens. To combat this issue, it is necessary to identify novel antibiotic producing bacteria. To do this, soil samples were collected from various locations on the campus of the University of Pittsburgh and tested for chemical and physical characteristics, since soil has historically yielded many widely used antibiotics. Bacteria from the soil were isolated on a variety of media, and tested against safe relatives of human bacterial pathogens that are most efficient at acquiring resistance and therefore are of the greatest danger. Antibiotic producers were then characterized and identified where possible. Organic extracts of cell metabolites from these isolates were also tested for antibiotic activity. SJR-R2A-5 and SJR-LBA-13 produced antibiotics against *Bacillus subtilis, Escherichia coli*, and *Erwinia carotovura*, while VMP-NA-16 was found to produce antibiotics against *Staphylococcus epidermidis*. SJR-R2A-5 is rod-shaped and was identified via BioLog analysis as *Pseudomonas*, a Gram negative bacterium. SJR-LBA-13 was found to be a Gram negative rod and was identified as *Pseudomonas* via 16S rDNA sequencing. VMP-NA-16 was found to be a Gram positive rod and was identified as *Brevibacterium* via 16S rDNA sequencing. Also, ethyl acetate extract of VMP-NA-16 cells inhibited *S. epidermidis*, further confirming this isolate's antibiotic production. Our soil samples contained cultureable bacteria that inhibit the growth of a variety of Gram positive and Gram negative bacteria.

18. More than Just D.I.R.T.T.: A Glimpse into the World of Antibiotic Resistance

Shannon Anderson, Audrey Black, Tori Gudmundsson, **Sara Held, Helena Hind, Lily Johnson**, Catherine Merrick, Cher Qin, Sabrina Sanchez, and Barbara Fishel

The Hockaday School, Dallas, TX

The Hockaday School, a preK-12, all-girls school in Dallas Texas, piloted The Small World Initiative in a novel year-long high school biology course. This poster reflects the students' developing understanding of the research process, presents an overview of a typical set of their experiments, and reveals their deepening recognition of the inherent connection between experimental results and the content covered in the classroom. Students state that the best parts of our partnership with SWI were that each experiment helped them learn more about biology. They comment that as their knowledge grew, and after multiple repetitions of experiments, their lab skills became more refined, teaching them patience within the context of their triumphs and failures, and leading to more successful results. Further, they report that SWI felt relevant as it informed them of the severity of the antibiotic resistance crisis, an issue which all now take seriously.

19. Bacteria Isolated from Soil Collected Near Eberly Hall Cultured on R2A Media Display Antibiotic Potential Against Relatives of Pathogenic Bacteria

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Novel antibiotics are increasingly in demand as antibiotic resistance lessens the effectiveness of medical treatment against bacterial infections. Bacteria in the soil, which naturally produce secondary metabolites, are a good source of these antibiotic compounds. In this study we aimed to isolate and identify antibiotic-producing bacteria from soil samples collected from the University of Pittsburgh campus. Serial dilutions of three soil samples were spread on LBA, R2A, 10% TSA, and NA media and incubated at 20˚C and 30˚C to maximize the growth of culturable bacteria. Bacterial isolates were screened for antibiotic activity against safe relatives of human pathogens. Select antibiotic-producing isolates were characterized by cell and colony morphology and identified by 16S rRNA gene sequencing. Antibiotic activity was also verified by an antibiotic screen of the organic extract. The three soil samples examined contained a maximum bacterial abundance ranging from 8.2×10^{6} to 1.15×10^{7}CFU/g. 7.81% of the isolates examined produced antibiotics against one or more tester strains. We characterized three isolates. Isolates AH-LBA-9 and MT-R2A-4 inhibited the growth of *B. subtilis* and were determined to be of the genus *Streptobacillus* and *Streptomyces*, respectively. Isolate MT-R2A-6 was found to be a *Staphylococcus* strain with antibiotic activity against *B. subtilis* and *S. epidermidis*. The organic extracts of isolates MT-R2A-4 and MT-R2A-6 also showed antibiotic activity. Our results have been recorded in the *Small World Initiative* database to help determine where antibiotic producers are best found. Our isolates will undergo further testing to determine the identity of their antibiotic compounds.

20. Antibiotic Initiative: Determination of Antibiotic Production of Bacteria Isolated from Soil Samples in Southeast Louisiana

Tiffany Brown, Tristan Leblanc, **Chantel Lee, James W. McCleary III**, and Mary G. Miller

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Research was conducted in an effort to fight the worldwide crisis of antibiotic resistance directed by the Small World initiative. Soil samples were collected from various locations throughout Southeast Louisiana. Bacterial colonies were isolated from each location and screened for antibiotic production against ESKAPE pathogens. Metabolic and morphological tests were performed on selected isolates. Identification of isolates was confirmed through sequencing and aligning of the 16S gene. This poster will be used to describe the three antibiotic producers found in this study, *Burkholdaria sp.*, *Bacillus pumilus*, *Brevibacillus laterosporus,* along with the one non-antibiotic producer, *Pseudomonas parafulva*.

TAPAS Presentations

- **Leveling down-SWI the Summer Camp Experience** presented by Dr. Michael Buckholt, Worcester Polytechnic Institute
- **SWI Biosafety Updates** presented by Dr. Kristen Butela, Seton Hill University
- **Eukaryotic testing to explore extract activity and foster inquiry** presented by Dr. Nichole A Broderick, University of Connecticut
- **Small World Initiative alluring Latin@s into Research** presented by Dr. Lilliam Casillas, Universidad de Puerto Rico-Humacao
- **The Microbial World: the benefits of an SWI course on non-science major students** presented by Dr. Debra Davis, Wingate University
- **More Than Just D.I.R.T.T: Piloting the SWI Hunt for Antibiotics in High School** presented by Dr. Barbara Fishel, The Hockaday School
- **Affordable whole–genome sequencing of 2 novel marine antibiotic producers** presented by Dr. Brittany J. Gasper, Florida Southern College
- **Antimicrobial Agents: What's in the Label** presented by Dr. Mary Miller, Baton Rouge Community College
- **High throughput antibiotic screening** presented by Dr. Mustafa Morsy, The University of West Alabama
- **The Isolation and Characterization of Marine Antibiotic Producing Bacteria** presented by Dr. Eric Warrick, State College of Florida
- **Identification of a Biosurfactant by way of the SWI** presented by Dr. Elizabeth Lewis Roberts, Southern Connecticut State University

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