SOC Antibiotic Bioprospecting Teacher Workshop

Cost: $1650/teacher **Dates:** March 4-7, 2023 (8am – 4pm daily)

Location: TBD

Instructors: Dr. Gabriel Vargas & Ibrahim Zuñiga (UW – Madison)

SOC Coordinator: Beth Hunt (BethHunt@soc-cr.org)

Seeds of Change's (SOC) mission is to provide programming to high school students that develops their critical thinking skills through original, experiential scientific research. SOC identified antimicrobial resistance as a compelling real-world health crisis that creative young minds could seek to solve through hands-on research. To support this aim, this course is designed train and certify teachers to run a bioprospecting research lab in their high school where students can learn how to discover novel antimicrobial candidates. SOC will support teachers/high schools who wish to establish a bioprospecting lab in their school with promotional lab equipment pricing, a repository for promising isolate storage, further isolate testing through SOC research partners, and shared intellectual property management.

Historically, bioprospecting efforts have focused on isolating antimicrobials from soil samples with a success rate on the order of 1 in 100,000. This training, however, focusses on learning how to isolate antimicrobial candidates that live symbiotically on and within insects. Research scientists are finding that bioprospecting using insect-derived samples has a much higher likelihood for success - on the order of 1 in 10-1000. SOC has 13 years of experience training students to develop original research projects focused primarily on leafcutter ant and butterfly insects. The goals of these experiential programs are to catapult each student into a scientific discovery mindset well before graduate school and in so doing motivate them to pursue education and careers in a STEM field.

This training course was developed in collaboration with SOC, Dr. Adrian Pinto's lab at the University of Costa Rica (UCR), Dr. Cameron Currie's lab at the University of Wisconsin - Madison (UW), Dr. Gabriel Vargas, Ibrahim Zuñiga, and Caitlin Carlson. In this course, teachers will learn how to:

- isolate bacteria strains from insect samples,
- establish isolate morphologies and other bioassay-determined characteristics,
- co-culture isolates for antimicrobial inhibition against antagonists
- use polymerase chain reaction (PCR) and electrophoresis to amplify and separate isolated genetic material by gene length,
- analyze 16S rRNA genetic sequence results to establish likely phylogenetic ancestry using MOLE-BLAST and MEGA, and
- learn how full genetic sequencing and antiSMASH identifies biosynthetic gene clusters (BGCs) that encode novel pathways for secondary metabolites with potential medical applications.

Potentially novel bioprospecting candidates identified in the training course and subsequent research by teachers and their students in high school bioprospecting labs can be sent for further analysis (Material Transfer Agreement required). Importantly, the high school, teachers and students that discover unique antimicrobial isolates will share in intellectual property rights with SOC and our collaborating partners.

To prepare for the workshop, please read: Novel Bioactive Natural Products from Bacteria via Bioprospecting, Genome Mining and Metabolic Engineering (Sekurova, Schneider, & Zotchev, 2019) http://bit.ly/SOC_NovelAMD. We recommend all researchers using Insect-Microbiome Antibiotic Bioprospecting protocols take the Microbiology Concept Inventory on Aseptic Techniques: http://bit.ly/AsepticWI.

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Bioprospecting Training Workshop Agenda

	Day 1	Day 2	Day 3	Day 4
Order Lunch				
Morning Session 9-11:30 am	Welcome AMR, Antibiotic Discovery & Insect Bioprospecting Insect ID & Apps LAB Insect Dissection & Plating (HW, larva wash, exoskeleton, gut & BB, CR gut) **11:45-noon Craig Kenesky, SOC Intellectual Property Consultant**	Bioinformatics LUCA ⇒16S ⇒IMG Instructor's research example application Gene alignment: BLAST & MOLE-BLAST LAB • Inoculate target organisms for co-cultures.	Load & Run electrophoresis gel wells MEGA – phylogenetic tree (Bess beetle example) Laptop MEGA phylogenetic tree guided work session	Characterizing Actinobacteria LAB More Insect Sample Plating (pupa & adult) LAB • Spore chain & Gram stain microscopy • Co-culture inhibition analysis and scoring Introduction to IMG/M
Lunch 12-1 pm				
Afternoon Session 1-4:00 pm (Day 4 ends at 3 pm)	Actinobacteria LAB • streak plate isolation • master patch plates from host plates. • motility & respiration assays	PCR & Primers Overview – avoiding contamination. Run PCR Issues with DNA extraction & Actinobacteria LAB Evaluate streak patch plates. Make agarose gels (GelRed™ with UV transilluminator)	1-1:15 John Doleman ZOOM, Bioprospecting Labs Discussions: Implementing Insect Bioprospecting Lab • Motility & respiration analysis Gene cluster research example AntiSMASH & BGCs Laptop AntiSMASH guided work	Student research examples from bioinformatics program. IMG/M guided activity (*Atta columbica*) Wrap up (Fig. 12): What you've accomplished! - - End 3 pm - -
HWK: < arrival: Read Novel Bioactive Natural Prods…	Read Evidence for Widespread Associations…	Read: Phylogenetic analysis of antibiotic-producing Streptomyces…	Read: The Antimicrobial Potential of *Streptomyces* from Insect Microbiomes	Post-workshop survey. Please complete within a week.

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What to Bring

1. Laptop that can download software (e.g., MEGA)
2. Personal Safety Goggles
3. A set of Micropipettors (0.5/1 uL – 10 uL, 10/20 uL – 100/200 uL, 100/200 uL – 1000 uL)
4. Water bottle

Workshop Accounts

There are several programs that we will interact with during the workshop that you need to establish accounts with and/or download prior to the workshop. The one that takes a few days is the IMG/JGI account.

Item	web address	Action	Directions
SOC IMAB workshop-related content for your use.	www.soc-cr.org	E-sign SOC IP agreement Create account Beth will approve Login upon approval	navigate to "contact us" menu item, click on "SOC Bioprospecting Login" Click on "Sign Up"
Dept. of Energy Joint Genome Institute (JGI) Integrated Microbial Genomes & Microbiomes (IMG/M) platform	https://img.jgi.doe.gov/cgi-bin/mer/main.cgi	Register account a week before workshop. JGI IMG/M will send you a notification when you are approved.	Click on JGI SSO Sign In button Click on "Register" button Fill in using your school as the "institution"
Molecular Evolutionary Genetics Analysis (MEGA 11) Software	https://www.megasoftware.net/	Download software	Navigate to MEGA website Select operating system (usually Windows or macOS) Select Graphical (GUI) Select MEGA 11 64-bit or MEGA 11 32-bit (depends on your computer's operating system)
Antibiotics and Secondary Metabolite Analysis Shell (AntiSMASH)	https://antismash.secondarymetabolites.org/	Bookmark website	Can download, but installation requires customization. The online version is adequate for our use.

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Reading List

This training was developed with guidance from SOC partner researchers at the University of Wisconsin - Madison and the University of Costa Rica. We recommend reading a few of their papers (whatever looks interesting to you!) and particularly the one on traditional and novel antibiotic bioprospecting methods (highlighted in yellow) as a lead into the workshop. Once at the workshop you will receive our 100+ teacher/student research manual that will provide context, protocols, and further resources.

Mechanisms of Antibiotic Resistance (Munita & Arias, 2016) http://bit.ly/SOC_AMR
Novel Bioactive Natural Products from Bacteria via Bioprospecting, Genome Mining and Metabolic Engineering (Sekurova, Schneider, & Zotchev, 2019) http://bit.ly/SOC_NovelAMD
An Ancient Ant-Bacteria Partnership to Protect Fungus (St. Fleur, 2018) http://bit.ly/SOC_NYT_Ant
Phylogenetic Analyses of Antibiotic-Producing Streptomyces sp. Isolates Obtained from the Stingless-bee *Tetragonisca angustula* (Apidae: Meliponini) (Cambronero-Heinrichs et al., 2019) http://bit.ly/SOC_StinglessB
Selvamicin, an Atypical Antifungal Polyene from Two Alternative Genomic Contexts (Van Arnam et al., 2016) http://bit.ly/SOC_Selvamicin
Evidence for Widespread Associations between Neotropical Hymenopteran Insects & Actinobacteria (Matarrita-Carranza, Currie, & Pinto-Tomas, 2017) http://bit.ly/SOC_NeoHym
The Antimicrobial Potential of *Streptomyces* from Insect Microbiomes (Chevrette et al., 2019) http://bit.ly/SOC_Streptomyces
The Sequence of Sequencers: The History of Sequencing DNA (Heather & Chain, 2016) http://bit.ly/SOC_DNAsequencing
Note: Green background indicates SOC Partner Research

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Workshop Instructors and SOC IP Consultant Bios

Dr. Gabriel Vargas: Gabriel has been the Lead Scientist for the Seeds of Change Bioinformatics program since its inception in 2014. This program teaches students how to run comparative analysis of genome and metagenome sequence data related to their unique research questions using the Integrated Microbial Genomes and Metagenomes database (IMG) and related tools. Recently he helped launch the Insect-Microbiome Antibiotic Bioprospecting Lab program by leading the pilot teacher training and helping develop the teacher training manual for the program.

Gabriel graduated from the University of Costa Rica with a degree in microbiology (2009) and stayed to earn his master's, also in microbiology (2014). He completed his doctorate in geophysical sciences at the University of Chicago (2020). Gabriel's research at the U Chicago Coleman lab focused on developing genetics tools to study the biogeochemistry and ecology of microbial aquatic systems. He developed an expertise in computational genomics through his research and has taught several workshops at the University of Costa Rica related to microbial multi-omics analysis and phylogenetics.

Gabriel is now a post-doctoral researcher with the Currie Lab at UW-Madison where he also collaborates with the Pinto Lab at the U. of Costa Rica. With a team of undergrads helping with isolations and bioassays, the Currie lab has over 30,000 isolated strains in their collection and over 100,000 co-culture inhibition assays.

Ibrahim Zuñiga: Ibrahim graduated from the University of Costa Rica with a degree in Microbiology (2013) and earn his Master's in Bacteriology in 2019. His undergrad research investigated amphibian populations that harbor antimicrobial producing bacteria in their skin to protect them against pathogens. For his master's degree Ibrahim studied how a family of beetles was only able to feed on wood through microbial cellulose digestion. Ibrahim is currently pursuing his PhD in the Suen lab at UW-Madison analyzing human gut microbiome data and its relation to health and disease. Ibrahim has worked with SOC as an Assistant Instructor for both the Tropical Field Research and Bioinformatics research immersion programs since 2015.

Dr. Craig Kenesky: Craig and his firm are providing intellectual property (IP) counseling to SOC, its teams, and alumni to help transform their scientific discoveries into valuable products. Craig is an associate in the New York office of Wilson Sonsini Goodrich & Rosati. Craig applies his background in synthetic organic, medicinal, peptide, bioorganic, and computational chemistry to the prosecution of domestic and foreign patent applications, as well as due diligence, freedom to operate, portfolio development strategy, opinions, and intellectual property counseling. Craig represents clients in numerous technology-focused industries, performs pre-transaction due diligence on behalf of both technology companies and venture capital groups, and he develops non-infringement and invalidity strategies for generic pharmaceutical companies. Craig teaches a class at Weill Cornell Medical College on the business strategies and decisions involved in the founding and early-stage management of a fundable and successful biotech start-up. Craig's doctoral research at the University of Pennsylvania focused on the total synthesis of architecturally complex anti-cancer natural products, inhibitors of HIV-1 protease, carbohydrate drugs, peptide-like biomaterials, and the computational and biochemical investigation of tubulin-drug interactions leading to cancer cell death.

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Workshop Contact Information

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*SOC Lead Teachers brought students on the SOC Tropical Field Research Program in Costa Rica		

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Non-pathogenic ESKAPE* Relatives Key

Non-pathogenic ESKAPE relative	#	Gram	Shape	Respiration	Motility	Indole	Spore forming
Bacillus cereus	1	+	rod (single or short chain)	fac. anaerobe	motile	-	yes
Staphylococcus epidermidis	2	+	cocci (grape-like clusters)	fac. anaerobe	non	+	non
Klebsiella (Enterobacter, Aerobacter) aerogenes	3	-	rod	fac. anaerobe	motile (peritrichous flagella – all sides)	-	non
Escherichia coli	4	-	rod	fac. anaerobe	Usually motile (peritrichous)	+	Non
*ESKAPE (See Table 14, P. 42)							

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Pre-Workshop Questions

(We will collect these and share with the instructors to help guide the workshop)

Today we will learn a bit of background on antimicrobial resistance and why Seeds of Change is collaborating with researchers focused on finding novel antibiotics from insect-bacteria symbiotic relationships. Later we will collect and aseptically plate samples from insects, which will involve dissection of live larvae and the use of micropipettors.

1. Are you comfortable dissecting/decapitating a live insect? Let us know any concerns you have.

2. Are you comfortable with aseptic techniques (e.g., using a flame and inoculation loop with Petri plates to transfer microbial material as a streak plate (Figure 42, P30)?

3. Are you comfortable with using micropipettors and the use of pipette tips (e.g., keep the pipette tip boxes closed to avoid contamination, how to adjust pipettes to the desired volume, how to dispense pipette volumes into vials)?

Before lunch we will do a quick zoom with Craig Kenesky an associate with Wilson Sonsini Patents & Innovations. Craig and his firm are providing intellectual property (IP) counseling to SOC, its teams, and alumni to help transform their scientific discoveries into valuable products. Review Figure 77 Isolate Decision Tree for Whole-genome Sequencing and SOC Partner Research (P52). Our program is designed to send promising isolates from high school labs to SOC partner researchers for further research that could ultimately evolve into a potential drug candidate. The initial isolation/discovery in your high school is the IP Seeds of Change seeks to manage on your behalf.

4. Think of a question you have related to IP that you might ask Craig if he doesn't touch on it.

On Day 2&3 you will prepare one or more PCR samples for thermocycling and gel electrophoresis.

5. How comfortable are you with the PCR/Electrophoresis process?
   - ☐ I can do it with my eyes closed
   - ☐ I do it once a year with my classes and reteach the details to myself annually
   - ☐ I don't use PCR/electrophoresis in my classes, but I understand the process, not the details
   - ☐ I'm not very familiar with PCR/electrophoresis – so will need extra guidance!

On days 3&4 we will introduce some computational biology/bioinformatics tools to you.

6. Please indicate which of the following you have experience with:
   - ☐ I (routinely, infrequently, never) have my classes do BLAST searches
   - ☐ I have used a bioinformatics tool (e.g., BLAST-MOLE, MEGA, other?) to make a phylogenetic tree.
   - ☐ I have used the Joint Genomics Institute's (JGI's) Integrated Microbial Genomes & Microbiomes platform before.
7. Circle the acronyms you are more than just familiar with: (PFAM, KEGG, BCGs, COG)

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Day 2 Warm-Up

Anti-microbial Resistance (AMR)

1. Explain how AMR is linked to vertical and horizontal gene transfer (Figure 6, P2).

Insect-Bacteria Symbiosis

2. Most antibiotics in use today were isolated from _____ (P6). SOC Partner Researchers have collected evidence that isolating samples from _____ is more likely to lead to novel antibiotics (P8). The transfer of symbiotic bacteria from one generation to another allows for more time and chances for more complex defense mechanisms to develop.

Insect Sample Preparation

3. What is PBS and why is it used in insect sample preparation (P25)?

4. What are two uses for glass microbeads (P27)?

5. What does YMEA stand for and what benefits do you see in its use in the classroom (P31)?

Bioinformatics

6. Explain the difference between genomes, metagenomes, and biosynthetic gene clusters (BGCs, P6).

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Day 3 Warm-Up

1. Navigate to the Howard Hughes Medical Institute Bacterial Identification Virtual Lab that walks you through polymerase chain reaction (PCR) and electrophoresis (A link to this virtual lab is in your IMAB manual on P. 47, last paragraph: http://bit.ly/SOC_PCR.) Click on "Launch Interactive" button and then click on the images/animations on the left side of the screen following the directions: Click to: "enter the lab", "on the glove box", etc.

2. 16S rRNA corresponds to a highly conserved gene responsible for _____ synthesis (P45). It is interspersed with hypervariable regions (V1-V9, Figure 67 & 69, P47 &48) of nucleotides (adenine, cytosine, guanine, and thymine: ACGT) that, in most cases, is enough genetic information to distinguish one _____ from another.

3. What's the distinction between bioinformatics and computational biology using bioinformatics tools (P57)? Which might you be doing in this training?

4. Metagenomics is the study of all the genetic material recovered directly from environmental sample (P. 43). One organism's complete set of DNA is called its _____.

5. Groups of genes that encode a multi-step, enzyme-catalyzed process or a biosynthetic pathway are called _____ _____ _____ or (BGCs, P6). Anti _ _ _ _ _ is a bioinformatics tool that allows researchers to determine % homology between BCGs in a genome of interest and known BCGs related to human health secondary metabolite production (P68).

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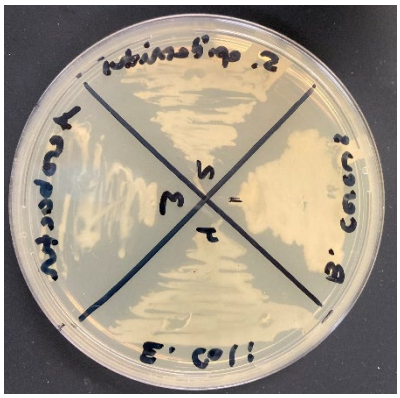
Day 4 Warm-Up

1. Assays that help characterize isolated bacteria, especially when compared to negative and positive controls - are inherently interesting. Thioglycollate broth filled test tubes are used to distinguish _____ (P36).

2. SIM semi-solid media with only 0.3% _____ allows for testing the _____ of a strain (P36). If the microbe reduces sulfur, the sulfur will react with the iron ion in the media and turn black. Whether the organism produces indole from tryptophane (indicated Enterobacter microbe) can also be determined if Kovac's agent is added to the media.

   Remembering to offer non-pathogenic, known microbes for students to compare assay results to is key.



3. The more sophisticated phylogenetic tree tool (__ __ __ __) allows you to (P63):
   - Run BLAST searches from within the program.
   - Select specific sequences to include.
   - Set alignment parameters. We recommend you use the _____ alignment method (p63).
   - Construct and display the tree (many options to adjust/alter to improve display).

4. During whole-genome sequencing, the extracted DNA is broken into millions of _____ - read fragments (p54). These fragments must be aligned and _____ into contigs and longer scaffolds to be useful for further analysis in antiSMASH or IMG/M (P54). Interestingly, even after this process is complete, you will not have the entire genome – there will be missing fragments.

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