GCAT-SEEK Educational Module – Jennifer A. Bennett and Kristen E. Giesting

Background

Triclosan is a synthetic antimicrobial commonly utilized in personal care products such as liquid handsoaps and toothpastes. Recently its use has been called into question based on numerous health and environmental studies (McMurry et al., 2004; Aiello et al, 2007; Dann and Hontela, 2010; Bertelsen, 2013; Marlatt et al., 2013; Ahn et al., 2008; Wu, Spongberg, Witter, 2009; McAvoy et al., 2002). We are interested in uncovering a possible correlation between the concentration of triclosan in the aquatic environment and the percentage of triclosan-resistant bacteria. Samples were taken from a wastewater treatment outfall, and sites immediately upstream and downstream as well as at the reservoir of a local stream. It is known that triclosan is not entirely removed during the treatment of wastewater, thus, it is expected that the chemical may be present in higher concentrations at the outfall site, possibly creating a selective pressure for triclosan resistant microbes (Dann and Hontela, 2010 and Sabaliunas et al, 2003). The use of genomics may reveal differences in microbial communities among the various sample locations.

Students will collect water samples and plate samples onto nutrient agar then streak colonies onto nutrient agar containing triclosan. Water samples will also be used directly to isolate genomic DNA using the Mo Bio Rapid Water Genomic DNA isolation kit. A library will be created using QIAGEN HOTSTAR and primers from IDT then sequenced using the Illumina MiSeq platform. Bioinformatics data will be analyzed using VirtualBox and QIIME (Caporaso et al, 2010). Phylogenetic trees will be created for each sample. Species diversity at the various sample sites will be compared to each other and the diversity among triclosan-resistant microbes will be related to that of the entire community.

Module Research Goals (Specific Aims)

1. Determine whether there is a correlation between the numbers of triclosan resistant bacteria and the concentration of chemical in the aquatic environment.
2. Compare species diversity among triclosan-resistant bacteria and overall species diversity.
3. Determine whether medically relevant bacteria are resistant to triclosan.

Learning Goals

By the end of this module, students will be able to:

1. Apply bioinformatics analyses to an environmental problem
2. Communicate experimental design and results
    a. Students will write a lab report
    b. Students will design an experiment for a future direction
3. Explain the impact of their actions on the environment and human health

    a. Community outreach presentation
4. Create phylogenetic trees

Vision and Change Core Competencies Addressed

1. Ability to apply the process of science
    a. Make a hypothesis
    b. Collect data
    c. Interpret data
    d. Present data - Create figures and synthesize supporting text for a lab report in the form of a scientific paper
    e. Design experiments as future directions
    f. Peer review
2. Ability to use quantitative reasoning
    a. Develop and interpret graphs
    b. Apply statistics
3. Ability to use modeling and simulation
    a. Apply bioinformatics tools and create phylogenetic trees
4. Ability to tap into the interdisciplinary nature of science
    a. Immunology and Analytical Chemistry to quantify triclosan concentrations
    b. Hydrology – Dynamics of water flow and impacts on properties of stream
    c. Statistics (Ex: applied to levels of resistant bacteria and diversity)
    d. Bioinformatics – Analyze species diversity using 16S rRNA sequencing
    e. Microbiology
    f. Molecular biology
    g. Medical field – antimicrobial resistance and cross-resistance to antibiotics
    h. Environmental Science
    i. Biochemistry/Microbial Physiology – Can relate species diversity to culturing and biochemical tests
5. Ability to communicate and collaborate with other disciplines
    a. Public relations/Communications Course
        i. Science students present to communications majors and both majors collaborate with each other to create a platform for social change
            1. Target groups on campus and/or the community
    b. Non-majors course (variety of non-scientific majors)
        i. Science students present to this class and class is asked to look for personal care products that contain triclosan
        ii. Understand how they can impact the environment and human health
    c. Education majors – Create lesson plans
6. Ability to understand the relationship between science and society

a. Education about the triclosan in consumer products and their impact on the environment and human health
b. Extensions of this project include cross-resistance to antibiotics and the examination of other environmental toxins and their impacts on human health

GCAT-SEEK Sequencing Requirements

- MiSeq is being used to provide the metagenomic data
- Other sequencing platforms that could be used include

Computer/Program Requirements for Data Analysis

- Computer: Mac or PC
- Program requirements: VirtualBox, QIIME

Assessment

- Attitude survey and pre-/post-test
- Submit a hypothesis for feedback
- Written lab report in the format of a scientific paper including figures and tables of data
- Peer review of lab report
- Oral presentation

Time Line of Module

This is a novel research project which examines the bacterial diversity in Alum Creek using 16S rRNA sequences from isolated bacteria to create phylogenetic trees. This project provides students with experience collecting water samples, plating for bacterial colonies, a variation on PCR (colony PCR using universal primers to 16s rDNA), sequencing, bioinformatics and phylogenetics. The data collected is anticipated to have an impact on the monitoring of Alum Creek.

Lab 1	Isolate bacterial samples from Alum Creek same day or class before, (Plate dilution series), Prepare genomic DNA directly from water sample
Lab 2	Colony counts; (Streak colonies onto triclosan plates or antibiotic plate of choice in a grid of 16 colonies per plate), Quantify genomic DNA prepared from previous lab using Qubit
Lab 3	PCR Reaction to Create Library for NextGen Sequencing; (Record numbers of resistant bacteria and put plates in the refrigerator until Lab 5)
Lab 4	Run PCR reactions on E-gel for confirmation of product, only 12 minutes to run or regular gel; examine gel picture; Use Qubit to quantify product and pool sample duplicates; Submit to core facility for MiSeq (or HiSeq) analysis

Lab 5	Gel purify PCR products and pool all samples to be run in sequencing lane; Submit to core facility for MiSeq (or HiSeq) analysis
Lab 6	(While waiting for sequencing data, colony PCR of resistant bacteria)
Lab 7	(Run PCR reactions on a gel and quantify to load 96-well plate for sequencing)
Lab 8	Begin to Analyze Sequences; (BLAST to identify resistant bacterial species)
Lab 9	Learn programming and clean-up data
Lab 10	Construct Phylogenetic trees for resistant microbes
Lab 11	Finish Phylogenetic Analysis (and compare diversity of whole community versus culturable, resistant bacteria within community)

*Module can be modified and shortened to only look at microbial diversity at each site, rather than relating this to antimicrobial/antibiotic resistance (optional antimicrobial steps in parentheses). It is also possible to analyze

Discussion Topics for Class

- Functional Genomics and Bioinformatics
- Next Gen
- Microbial Diversity
- Environmental Stewardship
- Antimicrobial Resistance
- Microevolution to produce resistant microbes
- Evolutionary relationships through the construction of phylogenetic trees
- Multidisciplinary nature of the project

Lecture Topics for Class

- Antibiotic/antimicrobial resistance
- Evolutionary relationships using 16S rRNA sequencing

References

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