Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

Project Manager Name: Timothy M. LaPara

Project Manager Email address: lapar001@umn.edu

Project Title: Triclosan impacts on wastewater treatment

Project number: 032-B

1. Abstract

The goal of this project is to understand the role of triclosan in selecting for antibiotic resistant bacteria during the municipal wastewater treatment process. The goal will be met by establishing numerous bench-scale wastewater treatment bioreactors fed a synthetic municipal wastewater containing different concentrations of triclosan. These bioreactors will then be compared on the basis of bacterial community composition (using a state-of-the-art ribosomal RNA approach), the quantities of several known antibiotic resistance genes (using quantitative polymerase chain reaction), the relatively quantities of all known antibiotic resistance genes (using shotgun metagenomics), and the presence of novel antibiotic resistance genes (using functional metagenomics). The proposed project, therefore, will fulfill a critical need in helping to define triclosan either as detrimental chemical with serious collateral effects or as a useful component of personal hygiene. The project will be directly beneficial to Minnesota's legislation as it considers action on triclosan and to Minnesota's citizens who currently use this chemical extensively.

2. Background

Antibiotics and antibacterials are used to protect the health of the general public from infectious bacteria. Antibiotics are naturally-produced compounds; in contrast, analogous but synthetically-produced compounds are called "antibacterials". Both antibiotics and antibacterials (the term "antibiotic" will be used to encompass both terms throughout the remainder of this proposal) specifically target a feature of the bacterial cell either to prevent cell growth (bacteriostatic) or to actively kill the cell (bacteriocidal). The specific nature of antibiotics is critical for medical purposes as it allows the drug to harm bacterial cells but not the patient's cells. This feature also distinguishes antibiotics from disinfectants, antiseptics, and other germicidal agents, which are typically effective against all cell types.

Unfortunately, antibiotic use, misuse, and overuse have served as selective pressures over the past 70 years, such that antibiotic resistant infections are frighteningly commonplace. The current "solution" to this problem is to limit further misuse and inappropriate use. For example, physicians are now strongly encouraged to forego prescribing antibiotics for common viral infections, such as influenza and the common cold, because antibiotic chemotherapy is entirely ineffective against viral infections. In addition, antibiotic use in agriculture has been banned in Europe, although antibiotic use in agriculture continues in the United States, but remains controversial.

The widespread use of triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) has also been identified as another misuse of antibiotics. Triclosan is an antibacterial agent used in numerous

commercial products, including liquid hand soap, toothpaste, cosmetics, and children's toys. This extensive use of triclosan could theoretically select for resistance to other antibiotics, as most antibiotics co-select for resistance to other antibiotics (O'Brien, 2002). Relatively little is known about the importance of triclosan in selecting for bacterial resistance to other antibiotics and what is available is often contradictory. There have been several clinical studies of the long-term effects of daily triclosan use in oral hygiene that demonstrated no increased level of resistance (McBain et al., 2004; Aiello et al., 2005). In contrast, investigations of intestinal bacteria have found that triclosan-resistant *E. coli* O157 strains showed decreased susceptibility to various other antimicrobials including ciprofloxacin, tetracycline, and others (Ledder et al., 2006).

The goal of this proposed project is to understand the role of triclosan in selecting for antibiotic resistant bacteria during the municipal wastewater treatment process. An emerging paradigm for thwarting the spread of antibiotic resistance is to enhance the nation's municipal wastewater treatment infrastructure (LaPara et al. 2011; Pruden 2013). The rationale for this paradigm is that people taking antibiotics will select for antibiotic resistant bacteria in their gastrointestinal tracts and then release these organisms upon defecation. This fecal material then coalesces at municipal wastewater treatment facilities where the treatment process could be used to eliminate antibiotic resistant bacteria. Because spent soap, toothpaste, etc., are washed down drains, municipal wastewater contains substantial concentrations of triclosan, thus exposing the antibiotic resistant bacteria from people's gastrointestinal tracts to yet another antimicrobial agent. Triclosan could, therefore, drive the selection of multiple antibiotic resistance during the wastewater treatment process, creating new bacterial strains that are resistant to numerous antibiotics — more commonly known as "super bugs" for their ability to resist many and potentially all antibiotics.

3. Hypothesis

The extensive use of triclosan and its subsequent accumulation in municipal wastewater treatment facilities selects for bacteria resistant to multiple antibiotics within the municipal wastewater treatment process.

4. Methodology

Experimental Approach. The proposed research will test the aforementioned hypothesis by operating bench-scale bioreactors fed a synthetic municipal wastewater containing four different concentrations of triclosan (a no-triclosan control, 1 μg/L, 5 μg/L, and 15 μg/L). Prior research has suggested that triclosan concentrations greater than 5 μg/L cause a statistically significant shift in bacterial community composition (Dr. Kris Wammer, University of St. Thomas, personal communication). The no-triclosan control bioreactors will be located in the laboratory of Dr. Bill Arnold at the University of Minnesota, who tightly controls the laboratory to ensure that no triclosan enters the laboratory, to ensure that these bioreactors remain triclosan-free. Triclosan levels will be monitored in all bioreactors using high performance liquid chromatography (HPLC) and a diode-array UV detector.

In general, the lab-scale bioreactors will simulate the activated sludge process, which is commonly used to treat municipal wastewater. A synthetic wastewater formulation will be used so the concentration of triclosan can be carefully controlled (genuine municipal wastewater is heavily contaminated with triclosan). Each of the experimental treatments will be performed in triplicate (4 conditions × 3 replicates = 12 bioreactors) to facilitate statistical analysis of the effects of triclosan on antibiotic resistance levels.

Sequencing Batch Reactors. Laboratory-scale wastewater treatment bioreactors will consist of 1-liter shake-flasks with a liquid volume of 200 mL and will be operated as sequencing batch reactors (SBRs). The SBRs will be operated at a hydraulic residence time of 32 hours and at a temperature of 25°C. The SBRs will be aerated by shaking at a rate of 150-200 rpm. On a daily basis, mixing will be stopped for 30 minutes and 150 mL of clear supernatant will be carefully remove using a glass pipette and replaced with fresh synthetic growth media. Biomass will also be removed from the shake-flasks to maintain a mean cell residence time of 8 days. SBRs will be inoculated with cryopreserved bacterial cells obtained from the aeration tank of a full-scale wastewater treatment facility. SBRs will be fed a synthetic growth medium consisting of 80 mg peptone, 80 mg tryptone, and 80 mg yeast extract per liter. The performance of these SBRs will be monitored by tracking dissolved oxygen concentrations, pH, the concentration of chemical oxygen demand (COD) in the treated wastewater, and the biomass levels (measured as dry cell mass). We anticipate operating these SBRs for 6-8 weeks, which exceeds the accepted "rule-of-thumb" that 3 to 5 mean cell residence times are required to establish stable performance. We anticipate collecting 7 to 10 samples from each of these SBRs throughout the experiment for subsequent analysis.

DNA Extraction and Purification. Samples for bacterial community analysis, quantitative PCR, and functional metagenomics will be collected as part of the biomass that is wasted daily from the SBRs to maintain the desired mean cell residence time. We anticipate requiring 1 mL of the SBR milieu to obtain enough biomass for these analyses. Samples for bacterial community analysis and qPCR will be centrifuged, the supernatant will be removed, and the cell pellet will be resuspended in lysis buffer (5% sodium dodecyl sulfate, pH = 8.0). The bacterial cells will then be lysed by performing three consecutive freeze-thaw cycles followed by a 90-minute incubation at 70°C. Community DNA will then be extracted and purified using a commercially-available kit. The LaPara laboratory has substantial experience performing these techniques (for example, see LaPara et al. 2011).

A similar approach will be used to obtain DNA for the shotgun metagenomic analysis, except that this biomass will be obtained at the end of the experiment. Shotgun metagenomics requires a relatively high quantity of DNA (3 μg of DNA in 25 μL of sterile water). We therefore anticipate collecting 50 mL of culture broth, centrifuging the sample to obtain a cell pellet, and then processing the sample as described above. We will collect replicate samples from each bioreactor to ensure that we have a sufficient quantity of DNA for analysis.

Bacterial Community Composition. The composition of the bacterial communities growing in the laboratory-scale SBRs will be tracked by using the polymerase chain reaction (PCR) to amplify the V3-region of the 16S rRNA gene, purifying these PCR products using a commercially-available kit, and then directly sequencing these PCR products using an Illumina MiSeq instrument. The output from the Illumina MiSeq data will be processed using Mothur (Schloss et al. 2009) to trim for sequence quality and to determine the diversity of the bacterial communities (Chao estimator, Shannon Index, etc.) and to identify the individual members of the bacterial community. The structure of the bacterial communities will be statistically compared by non-metric multidimensional scaling (nMDS) as described previously (Nelson et al. 2010).

The approach used will be similar to that described by Bartram et al. (2011) except that we will multiplex as many as 144 samples simultaneously (i.e., we anticipate completing this task with a single run of the MiSeq instrument). The LaPara laboratory has recently multiplexed 96 different bacterial communities; we anticipate that the bacterial communities from our bench-scale SBRs will have very limited diversity/complexity, such that multiplexing large numbers of samples will be easily accommodated. We anticipate that bacterial communities grown at 15 μg/L will have a significantly different composition than the bacterial communities grown at lower

concentration. This will suggest that triclosan has a significant effect on the bacterial community composition at these concentrations.

Quantitative PCR. Quantitative PCR (qPCR) will be used to quantify three genes that encode resistance to tetracycline (*tet*(A), *tet*(W), and *tet*(X)) as well as genes that encode for resistance to macrolides (*erm*(B)), sulfonamides (*sul1*), and fluoroquinolones (*qnrA*). In addition, qPCR will be used to quantify the 16S rRNA gene (a measure of bacterial biomass), the integrase gene of Class 1 integrons (*intI1*), and the origin of replication of IncA/C plasmids (*repA*). Class 1 integrons are genetic constructs that more easily allow bacteria to incorporate exogenous genetic material and modulate its expression (Mazel, 2006); integrons are typically associated with multiple antibiotic resistance. IncA/C plasmids are highly conserved plasmids found in many human and animal pathogens; IncA/C plasmids contain multiple genes encoding antibiotic resistance. The LaPara laboratory has substantial experience performed qPCR targeting each of these genes (for example, see Burch et al. 2013).

We anticipate performing qPCR on 84 samples (samples collected on 7 different dates from 12 different SBRs) for each of the aforementioned resistance genes. From a practical perspective, we can quantify about 84 samples on a single run on the real-time PCR instrument; this instrument contains 96 wells, but we use about a dozen of these wells for standards and negative controls. We anticipate that the bacterial communities growing at higher concentrations of triclosan will contain greater quantities of the antibiotic resistance genes.

Shotgun Metagenomics. Shotgun metagenomics will be performed on the three bacterial communities grown in the absence of triclosan and the three bacterial communities grown in the presence of 15 μg/L of triclosan (we will attempt to sequence additional bacterial communities if time and the budget allows). Metagenomic DNA will be sequenced using Illumina MiSeq instrument at the University of Minnesota Genomics Facility (UMGC). We anticipate using six different runs on the MiSeq instrument (i.e., no multiplexing) to generating a very large quantity of DNA sequence data from each sample; the LaPara lab, for example, was able to generate > 30× coverage of 24 different bacterial genomes that were multiplexed and sequenced simultaneously.

The metagenomic DNA sequence data will be initially trimmed for quality and for size using Mothur. Assembly will be performed with khmer for digital normalization (Zhu et al., 2010), followed by assembly with velvet (Zerbino and Birney, 2008) and annotation of protein-coding genes using MetaGeneMark (Zhu et al., 2010), SEED Subsystems (Overbeek et al., 2005), and USEARCH (Edgar, 2010). The annotated protein sequences from each sample will be clustered for multiple sample ordination in QIIME (Caporaso et al. 2010). The presence of genes of interest (e.g., ARGs) also will be assessed via the Comprehensive Antibiotic Resistance Database (CARD) (McArthur et al., 2013). This approach will allows us to discern differences among gene types between samples, specifically antibiotic resistance genes in this case. Analysis of the metagenomic data will be performed in collaboration with Dr. Jeff Werner from the State University of New York at Cortland.

These experiments are expected to show the three cultures grown at triclosan concentrations of 15 μg/L will have a greater diversity and a greater quantity of ARGs. This approach will be particularly powerful as it will allow us to track all known ARGs simultaneously (albeit at a lesser quantitative precision than provided by qPCR).

Functional metagenomics. Whereas the previous approaches will be capable of precisely quantifying a handful of known ARGs (via qPCR) or detecting all known ARGs (via shotgun metagenomics), it is generally assumed that only a small fraction of all ARGs have been

identified to date. We will, therefore, use a functional metagenomics to detect and to identify novel ARGs in the bacterial communities grown in the presence of triclosan and the bacterial communities grown in the absence of triclosan. The Donato Laboratory at the University of St. Thomas has substantial experience using this technique.

Samples from the no-triclosan control SBRs and from the bacterial communities grown at a triclosan concentration 15 μg/L will be used for these experiments. Fosmid libraries will be constructed from each sample as previously described (Donato et al. 2010). Briefly, cells will be isolated and embedded in agarose plugs (1.4% low melt agarose, 1× TAE buffer). The agarose and cells will be incubated in lysis buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 0.2 M EDTA pH 8.0, 1% (w/v) sarkosyl, 1% (w/v) sodium deoxycholate, 1 mg/mL lysozyme). After incubation at 37°C for three hours, the lysed cells will be washed with ESP Buffer (1% (w/v) sarkosyl, 0.5 M EDTA pH 8.0, 1 mg/ml proteinase K). After two overnight washes at 55°C, the cells will be soaked in $T_{10}E_1$, and the DNA will be size-selected via pulse-field gel electrophoresis. DNA that is 40-100 kb in length will be excised from the gel and will be purified from the agarose using Gelase (Epicentre Biotechnologies) according to the manufacturer's protocol. The resulting DNA will be blunt-ended, ligated into the multiple cloning site of the pCC1FOS Copy Control Fosmid vector, and introduced into *E. coli* strain *EPI300* according to the manufacturer's protocol (Epicentre Biotechnologies). After growing the library on LB agar supplemented with chloramphenicol (20 μg/mL) to select for clones, the library will be harvested and stored in LB supplemented with 10% glycerol at -80 °C.

The selection of clones bearing antibiotic resistance genes will be done as previously described (Donato et al. 2010). Briefly, library aliquots will be removed from the freezer, and will be grown in LB supplemented with chloramphenicol (20 μg/mL) at 37°C for two hours. Aliquots of each culture will be diluted in water and plated on selective media at a concentration of 10^5 to 10^6 cells per plate. The selective media will include LB agar supplemented with the following antibiotics: ampicillin (50 μg/mL), ceftazidime (1 μg/mL), carbenicillin (50 μg/mL), tetracycline (10 μg/mL), kanamycin (20 μg/mL), ciprofloxacin (concentration to be determined empirically), and meropenem (concentration to be determined empirically). Plates will be incubated at 37°C overnight. For the antibiotics whose concentration is yet to be determined, a minimum inhibitory concentration (MIC) assay will be conducted using the host strain of *E. coli* according to the serial dilution method. The results of those assays will enable a choice of antibiotic concentration that is sufficiently above the MIC to eliminate false positives, but not so far above the MIC that it will prevent growth of all cells. Colonies that appear on the selective media will be retained for further analysis.

Cells harboring clones that grow on media containing antibiotics will be verified by reintroduction into fresh cells, followed by reselection for increased tolerance to the appropriate antibiotic. All fosmid clones that successfully lead to growth on media supplemented with antibiotics will be subjected to restriction fragment length polymorphism (RFLP) analysis to facilitate grouping of clones with inserts from identical organisms. One clone from each RFLP group will be purified and submitted for Illumina MiSeq sequencing. Because these fosmids are relatively small (30-50 kb), we anticipate being able to multiplex hundreds of clones. Sequences will be trimmed for quality using Mothur and assembled using Velvet. Fosmids will then be annotated manually; unique and interesting fosmids (i.e., those containing putatively novel genes) will be subcloned into the pET28(b) expression vector to facilitate purification and subsequent biochemical characterization of the enzymes responsible for the observed resistance.

We anticipate that the bacterial communities growing in the presence of 15 μg/L of triclosan will contain numerous ARGs. In contrast, the bacterial communities growing in the absence of triclosan will contain few novel ARGs.

5. Results and Deliverables

The primary goal of the proposed research will be to determine whether triclosan selects for bacterial resistance to other antibiotics, as is typically assumed in the scientific literature. This goal will be achieved by growing bacteria at different concentrations of triclosan (using a model system that mimics wastewater treatment bioreactors) and then comparing the composition of these bacterial communities in four different ways: (1) the identities of members of the bacterial communities by targeting 16S rRNA gene fragments, (2) the quantities of several, known antibiotic resistance genes, (3) the metagenomic DNA composition – this will be used to investigate the presence/absence of all known antibiotic resistance genes, and (4) the presence of previously unknown antibiotic resistance genes using a functional metagenomic approach. The deliverables from the proposed project will include the reports submitted to the LCCMR as well as peer-reviewed publications. We also anticipate presenting our research results at local, regional, national, and international conferences.

6. Timetable

The research tasks outlined above will be accomplished according to the following schedule. Shaded regions are continuous efforts and ×'s mark discreet events.

	Year 1				Year 2				Year 3			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Activity 1: SBR operation and sample collection												
Inoculate SBRs	×											
Sample Collection												
End SBR operation		×										
Activity 2: Characterization of SBR communities												
DNA extraction and purification												
Community analysis of 16S rRNA genes												
qPCR targeting known ARGs												
Metagenomic analysis of communities												
Data Analysis												
Activity 3: Functional metagenomic analysis of SBR communities												
Fosmid library construction												
Screening of clones												
DNA sequencing of specific clones												
Reporting		×		×		×		×		×		×

7. Budget

The proposed research project and related report preparation will require 36 months to complete, and therefore 36 months of support is requested for this project. The project will be performed at both the University of Minnesota (this portion will directed by Dr. Timothy M. LaPara) and the University of St. Thomas. The total budget for the proposed project is $380,000, of which $244,217 will support work at the University of Minnesota and $135,783 will support work at the University of St. Thomas (via a subcontract from the University of Minnesota). The justification for the items specifically listed within the requested budget is presented below.

Senior Personnel. Dr. LaPara will jointly direct the research proposed herein with Dr. Donato from the University of St. Thomas. Dr. LaPara's responsibilities will include laboratory training of the graduate student at the University of Minnesota, report preparation, and co-authoring of papers to publish the research findings in peer-reviewed journals of scientific merit. Salary is requested for Dr. LaPara's participation on this project (1.25 months in the years 1 and 2; 0.5 months in year 3). Dr. Donato's responsibilities will include laboratory training of the undergraduate students at the University of St. Thomas, report preparation, and co-authoring of papers to publish the research findings in peer-reviewed journals of scientific merit. Salary is requested for Dr. Donato's participation on this project (1.5 months each year).

Other Personnel. One Ph.D. student at the University of Minnesota will be supported by this project as a research assistant. The graduate student will be supported as a 50% research assistant during all three years of the project. The graduate student will perform the proposed experiments, co-author research manuscripts, and present data at conferences. Five undergraduate students will be supported on an hourly basis at the University of St. Thomas. These students will be responsible for performing the proposed experiments; they will also help co-author research manuscripts and present data at conferences, if they are interested in doing so. Undergraduate students will be supported part-time during the academic year and full-time during the summer months.

Fringe Benefits. Fringe benefits costs will be incurred for Dr. LaPara (University of Minnesota faculty fringe: 33.6%) and the graduate student (health care: 15.7% of salary; summer FICA: 7.4%; and tuition). Undergraduate students at the University of St. Thomas will also have fringe benefits costs for their summer salary (summer FICA).

Travel. A total of $1,500 of travel funds for travel from the University of Minnesota is requested. A portion of these travels funds will be used to travel to various wastewater treatment facilities to collect samples. This money will be used for renting cars and reimbursing for personal automobile use. Other funds will be used to attend in-state conferences to present our research results.

Materials and Supplies. A total of $56,400 is requested for materials and supplies to perform the proposed project. This money will be used to purchase expendable supplies such as chemicals, reagents, and glassware. Substantial portions of the costs will be for DNA extraction kits, cloning kits, DNA purification kits, protein purification kits, growth media, antibiotics, *E. coli* cells, and supplies for quantitative PCR.

Other. This project will incur substantial expenses ($54,329) for DNA sequencing and related other manipulations (e.g., sheering and tagging of DNA as preparation for Illumina sequencing, data analysis) from the University of Minnesota Genomics Center (UMGC) and from the Minnesota Supercomputing Institute. UMGC provides equipment and technicians at cost for University of Minnesota researchers. We anticipate using numerous runs of the Illumina MiSeq

and instruments, which range in cost from $1000 to $2000 per run, to perform the metagenomic and functional metagenomic analysis proposed herein. We will also incur costs for purchasing PCR primers and DNA shearing and other manipulations performed by UMGC. Finally, this research will generate terabytes of DNA sequence information; this data with be stored at the Minnesota Supercomputing Institute at a nominal cost.

In-Kind Services. Neither the University of Minnesota nor the University of St. Thomas will charge overhead costs on this project. The federally-negotiated overhead rate for the University of Minnesota is 52%; we therefore estimate the in-kind contribution to be *at least* $127,000.

8. Credentials

Dr. LaPara is a nationally-known researcher with substantial research experience in the areas of environmental biotechnology, microbial ecology, and environmental microbiology. He is one of the leading researchers on municipal wastewater treatment and its potential role in thwarting the spread of antibiotic resistance. He has substantial expertise in quantifying ARGs in the environment, from both aquatic and sediment samples. He has co-authored more than 50 manuscripts in the peer-reviewed archival literature that have been cited more than 1000 times according to Web of Science.

Dr. Donato is prominent researcher in the field of functional metagenomics; having received his postdoctoral training in the laboratory of Jo Handelsman, the founder of the field. He has broad experience bridging the fields of environmental microbiology, antibiotic resistance, microbial genetics, functional metagenomics, and biochemistry. His most recent publications have focused on the identification and characterization of previously unknown antibiotic resistance genes from environmental samples.

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Professional Preparation
University of Notre Dame, Department of Civil Engineering and Geological Sciences, B.S.C.E., 1995.
Purdue University, School of Civil Engineering, Ph.D., 1999

Appointments
2013-present **Professor**, Department of Civil Engineering, University of Minnesota
2006-2013 **Associate Professor**, Department of Civil Engineering, University of Minnesota
2000-2006 **Assistant Professor**, Department of Civil Engineering, University of Minnesota

Professional Societies
International Water Assocation (IWA), Water Environment Federation (WEF), International Society for Microbial Ecology (ISME), American Society for Microbiology (ASM), American Society of Civil Engineers (ASCE)

Selected Peer-Reviewed Publications
Five most closely related to the proposed project:
1. Burch TR, MJ Sadowsky, and TM LaPara. 2013. Air-drying beds reduce the quantities of antibiotic resistance genes and class 1 integrons in residual municipal wastewater solids. *Environmental Science and Technology* **47**(17):9965-9971.

2. Ling AL, N Pace, MT Hernandez, and TM LaPara. 2013. Tetracycline resistance and Class 1 integron genes associated with indoor and outdoor aerosols. *Environmental Science and Technology* **47**(9):4046-4052.
3. Burch TR, MJ Sadowsky, and TM LaPara. 2013. Aerobic digestion reduces the quantity of antibiotic resistance genes in residual municipal wastewater solids. *Frontiers in Microbiology – Antimicrobials, Resistance and Chemotherapy* **4**:17. doi: 10.3389/fmicb.2013.00017.
4. LaPara TM, TR Burch, PJ McNamara, DT Tan, M Yan, and JJ Eichmiller. 2011. Tertiary-treated municipal wastewater is a significant point-source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science and Technology* **45**(22):9543-9549.
5. Diehl DL, and TM LaPara. 2010. Effect of temperature on the fate of genes encoding tetracycline resistance and the integrase of class 1 integrons within anaerobic and aerobic digesters treating municipal wastewater solids. *Environmental Science and Technology* **44**(23):9128-9133.

Five other products:

1. Wunder DB, DT Tan, TM LaPara, and RM Hozalski. 2013. The effects of antibiotic cocktails at environmentally relevant concentrations on the community composition and acetate biodegradation kinetics of bacterial biofilms. *Chemosphere* **90**(8):2261-2266.
2. Nelson DK, TM LaPara, and PJ Novak. 2012. Structure and function of assemblages of Bacteria and Archaea in model anaerobic aquifer columns: Can functional instability be practically beneficial? *Environmental Science and Technology* **46**(18):10137-10144.
3. Nelson DK, TM LaPara, and PN Novak. 2010. Effects of ethanol-based fuel contamination: Microbial community changes, production of regulated compounds, and methane generation. *Environmental Science and Technology* **44**(12):4525-4530.
4. Ghosh S, SJ Ramsden, and TM LaPara. 2009. The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants. *Applied Microbiology and Biotechnology* **84**(4):791-796.
5. Zhang P, TM LaPara, EH Goslan, Y Xie, SA Parsons, and RM Hozalski. 2009. Biodegradation of haloacetic acids by bacterial isolates and enrichment cultures from drinking water systems. *Environmental Science and Technology* **43**(9):3169-3175.

Synergistic Activities

- Editorial Board, *FEMS Microbiology Ecology* (2013-date)
- Editorial Board, *Applied and Environmental Microbiology* (2007-date)
- Associate Editor, *Journal of Environmental Engineering* (2008-date)
- Faculty Mentor, Minnesota Environmental Engineers, Scientists and Enthusiasts (2001-date)
- Undergraduate Research Mentor (22 students total; 2001-date)

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Professional Preparation

University of Delaware, Chemistry, Concentration in Environmental Chemistry, Minor in Mathematics, B.S., 1999
Cornell University, Biochemistry, Molecular, and Cell Biology, Minor in Genetics, Ph.D., 2006

Appointments

2010-present **Assistant Professor**, Department of Chemistry, University of St. Thomas

2011-present **Chair**, Biochemistry Program, University of St. Thomas
2006-2010 **Research Associate,** Department of Bacteriology, University of Wisconsin – Madison

Professional Society
American Chemical Society (ACS)

Selected Peer-Reviewed Publications
Five most closely related to the proposed project:
1. Shade A, AK Klimowicz, RN Spear, M Linske, JJ Donato, CS Hogan, PS McManus, and J Handelsman. 2013. Streptomycin application has no detectable effect on bacterial community structure in apple orchard soil. *Applied Environmental Microbiology* **79**(21): 6617-6625.
2. Donato, JJ, MA Klimstra, JR Byrnes, RJ White, and TC Marsh. 2012. The Introduction of Metagenomics into an Undergraduate Biochemistry Laboratory Course Yielded a Predicted Reductase that Decreases Triclosan Susceptibility in *E. coli*. *DNA and Cell Biology* **31**(6): 968-973.
3. Donato, JJ, LA Moe, BJ Converse, KD Smart, FC Berklein, PS McManus, and J Handelsman. 2010. Functional metagenomics reveals antibiotic resistance genes encoding bifunctional proteins in apple orchard soil. *Applied Environmental Microbiology* **76**(13): 4396-4401.
4. Rios-Velazquez, C, LL Williamson, KA Cloud-Hansen, HK Allen, MD McMahon, ZL Sabree, JJ Donato, and J Handelsman. 2011. Summer workshop in metagenomics: One week and eight students equals gigabases of new information. *Journal of Microbiology and Biology Education* **12**(2):120-126.
5. Allen, H.K., J Donato, HH Wang, KA Cloud-Hansen, J Davies, and J Handelsman. 2010. Call of the wild: Antibiotic resistance genes in natural environments. *Nature Reviews Microbiology* **8**(4):251-259.

Five other products:
1. Keich U, H Gao, JS Garretson, A Bhaskar, I Liachko, JJ Donato, and BK Tye. 2008. Computational detection of significant variation in binding affinity across two sets of sequences with application to the analysis of replication origins in yeast. *BMC Bioinformatics* 9:372-383.
2. Donato, JJ, SCC Chung, and BK Tye. 2006. Genome-wide hierarchy of replication origin usage in *Saccharomyces cerevisiae*. *PLoS Genetics* **2**(9):1328-1338.
3. Chang, VK, JJ Donato, CS Chan, and BK Tye. 2004. Mcm1 promotes replication initiation by binding specific elements at replication origins. *Molecular and Cellular Biology* **24**(14): 6514-6524.
4. Fitch, MJ, JJ Donato, and BK Tye. 2003. Mcm7, a subunit of the presumptive MCM helicase, modulates its own expression in conjunction with Mcm1. *Journal of Biological Chemistry* **278**(28):25408-25416.
5. Chang, VK, MJ Fitch, JJ Donato, TW Christensen, AM Merchant, and BK Tye. 2003. Mcm1 binds replication origins. *Journal of Biological Chemistry* **278**(8):6093-6100.

Synergistic Activities
- Outside project advisor to Dr. Hetty Blaak, National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Netherlands Laboratory for Zoonoses and Environmental Microbiology (2010-present)
- Peer reviewer for the following journals: *BMC Microbiology, PLOS ONE, Environmental Science and Technology, DNA and Cell Biology*, and *FEMS Microbiology*

- Peer reviewer of grant proposals submitted to the following agencies: (NERC) Natural Environmental Research Council (United Kingdom), (COST) European Cooperation in Science and Technology through the Federal Department for Homeland Affairs (Switzerland), and (RIVM) National Institute for Public Health and the Environment (Netherlands)
- Peer reviewer and grant panel member National Institute of Environmental Health Sciences (2013)
- Undergraduate research advisor to 18 students (2010-present)
- MN State Science Bowl Volunteer (2010-present)

9. Dissemination and Use

Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. We will also, when appropriate, disseminate results via press releases to the media. The audience is not only the scientific community, but also the public, policymakers, and practitioners. The work will also be of interest to the medical community and we will seek avenues to share the results with this community.

10. References

1. Aiello AE, B Marshall, SB Levy, P Della-Latta, SX Lin, and E Larson. 2005. Antibacterial cleaning products and drug resistance. *Emerging Infectious Diseases* **11:**1565-1570.
2. Bartram AK, MDJ Lynch, JC Stearns, G Moreno-Hagelsleb and JD Neufeld. 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Applied and Environmental Microbiology* **77:**3846-3852.
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