**California Sea Grant College Program**
**Final Report**

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Transferrable antibiotic resistance plasmids in urban coastal wetlands

David E. Cummings
619-849-2642
davidcummings@pointloma.edu

Eva Top
208-885-5015
evatop@uidaho.edu

**Project Hypothesis**
The overarching hypothesis driving this research is that antibiotic resistance genes released into the natural environment through urban storm water may persist, creating a reservoir of resistance genes that have the potential to return to the human community through various vectors such as birds, insects, and fish. The specific aim of this Program Development grant is to assess the diversity of multidrug-resistance plasmids in sediments of two urban wetlands.

**Project Goals and Objectives**
1. Isolate resistance plasmids from sediments of urban wetlands using bi- and tri-parental mating techniques.
2. Determine resistance profiles of isolated plasmids by disk diffusion technique.
3. Infer plasmid host range, and thus likelihood of horizontal transfer, from incompatibility groups using PCR-based replicon typing.

**Briefly describe project methodology**
Plasmid isolation. Live wetland bacteria were collected by dislodging them from sediment particles with tetrasodium pyrophosphate. Biparental matings consisted of wetland bacteria mixed with azide-resistant *E. coli* J53 AzR or rifampicin-resistant *P. putida* KT2442, immobilized on a polycarbonate filter to encourage conjugation. Transconjugants were selected with tetracycline (to select for plasmids) and either sodium azide or rifampicin. Tri-parental matings were carried out with wetland bacteria mixed with two lab strains: rifampicin-resistant *E. coli* HY842 and *E. coli* DH5alpha carrying mobilizable plasmid pSU4814 conferring chloramphenicol resistance. Transconjugants were selected with rifampicin and chloramphenicol.

Plasmid screening. Putative transconjugants were subjected to plasmid mini-preps to verify plasmids and RFLP digestion to identify distinct plasmids.

Resistance profiles. Transconjugants were subjected to antibiotic disk diffusion testing to determine the resistance phenotypes conferred by the environmental plasmids.

PCR-based replicon typing. Plasmid incompatibility groups were identified with multiplex PCR using primers targeting specific replicon-specific genes.

**Describe progress and accomplishments toward meeting goals and objectives.**

Goal 1. Isolate resistance plasmids from sediments of urban wetlands using bi- and tri-parental mating techniques.

In May 2011, PI Cummings and undergraduate student Doug Zuill travelled to the University of Idaho to learn the bi-and tri-parental mating techniques in the lab of collaborator Dr. Eva Top. Zuill spent two weeks at Idaho working alongside a PhD student applying the methods to the Moscow, Idaho wastewater outfall. Upon returning to PLNU, Zuill adapted the methods to wetland sediments. Fifteen distinct plasmids were isolated from the Tijuana River Estuary and Famosa Slough, San Diego County, using the bi- and tri-parental mating techniques. All 15 of the plasmids are self-transmissible and pose a threat to transfer horizontally to other bacteria.

2. Determine resistance profiles of isolated plasmids by disk diffusion technique.

By the nature of the bi-parental mating technique, which requires selection of plasmids with an antibiotic, those isolated by bi-parental mating were resistant to at least tetracycline. The resistance profiles of all 15 plasmids are under investigation using the disk diffusion technique. The first plasmid to be characterized in this study was pTRE.P11, isolated by the bi-parental mating method. pTRE.P11 conferred resistance to the following antibiotics to its surrogate host, P. putida KT2442: tetracycline, doxycycline, ampicillin, nalidixic acid, streptomycin, and sulfamethoxazole/trimethoprim. Antimicrobial susceptibility testing is in progress for the remaining 14 plasmids.

3. Infer plasmid host range, and thus likelihood of horizontal transfer, from incompatibility groups using PCR-based replicon typing.

pTRE.P11 was PCR-positive for the broad host-range IncP incompatibility group. PCR-based replicon typing is underway for the remaining plasmids.

**PROJECT MODIFICATIONS:**

None.

**PROJECT OUTCOMES:**

Cummings and Top submitted a pre-proposal to Sea Grant to continue this work. However, we were not encouraged to submit a full proposal. We are now preparing a full proposal for the NIH.

Results from this work will be submitted for publication when sufficient data are collected.

**IMPACTS OF PROJECT:**

The most direct impact of this short project was the training of an undergraduate student in a Tier I research lab. This student is now planning to pursue a PhD in wetland microbiology.

It is our sincere desire that the results from this work will eventually influence the public health and wastewater treatment communities.

**BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS:**

None as yet.

**ECONOMIC BENEFITS generated by discovery**

N/A

**Issue-based forecast capabilities**
N/A

**Tools, technologies and information services developed**
None as yet.

**Publications**
None from the PD grant in question, though there have been previous articles written at PLNU and CASG on this work.

**DISSEMINATION OF RESULTS:**
There are no presentations or publications resulting directly from this brief study. However, with previous CASG PD grants the PI has published 2 journal articles and is the process of writing an invited review article on the subject; and multiple public presentations including local and national conferences.

**COOPERATING ORGANIZATIONS:**
Federal
US Fish and Wildlife Services
NOAA - TRNERR

State
City of San Diego
CA State Parks

Nongovernment
Southwest Wetlands Interpretive Association

International
None

Industry
None

Academic
Point Loma Nazarene University
University of Idaho

Sea Grant
None

Other Organizations
None

**INTERNATIONAL IMPLICATIONS:**
The Tijuana River Estuary is a Wetland of International Importance (Ramsar site # 1452) and has an important role in the ecological health of coastline in both the US and Mexico.

**AWARDS:**
none

**KEYWORDS:**
wetlands, microbiology, antibiotic resistance, plasmids, DNA

**PATENTS:**
none

**FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:**
Volunteer Count 0
Graduate Student Info: N/A