AMERICAN SOCIETY FOR MICROBIOLOGY

TEXAS BRANCH FALL MEETING

October 18-20, 2012

Baylor University • Waco, Texas

BAYLOR UNIVERSITY

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ACKNOWLEDGEMENTS

The ASM Texas Branch extends special thanks to the Baylor University Department of Biology, College of Arts and Sciences, Baylor Event Services, and BURST.

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Marvin Whiteley (UT Austin) 2009-2011

SCHEDULE

THURSDAY EVENING OCTOBER 18, 2012

5:00- 7:00 Registration/Check-in
BSB E wing 1st floor Atrium

7:15-8:30 Welcome and Plenary / BSB B110
Strategies to Dissect Tuberculosis Pathogenic Mechanisms
Dr. Jeffrey D. Cirillo, Professor, Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center Bryan, TX

FRIDAY OCTOBER 19, 2012

7:00-8:15 Registration, Breakfast and Vendors
BSB Atrium and 2nd Floor E Wing Elevator Landing

8:15-8:30 Welcome/BSB B110

8:30- 9:30 General Microbiology Sessions/B110
8:30-8:45 Cheramie Trahan (FACULTY)
8:45- 9:00 Marnie Rout (POSTDOC)
9:00-9:15 Elizabeth Franks
9:15-9:30 Mary Weber

9:30-10:00 Coffee Break /2nd Floor E wing Elevator Landing

10:00-11:00 General Microbiology Sessions (Concurrent Sessions)

Session A D109
- 10:00 Beach-Letendre
- 10:15 Bardwell
- 10:30 Visi
- 10:45

Session B D110
- 10:00 Alam
- 10:15 Myagmarjav
- 10:30 Okjay
- 10:45 Lal

11:00 a.m.-12:00 p.m. Meet the Vendors/First floor Atrium

12:00-1:00 p.m. Lunch/Poster Set Up

1:00-2:30 p.m. Poster Judging/2nd floor Middle Corridor

SCHEDULE

2:30-3:30 Goldschmidt Award Lectures

Dr. Millicent Goldschmidt has been instrumental in making the Texas Branch one of ASM's strongest branches. Each year she supports our branch with a monetary award for our future microbiologists. This year award winners, Holly Huse and Miry Girard will present information about their training, research experiences, and future goals.

2:30- 3:00 Holly Huse/B110
Department of Molecular Genetics and Microbiology, The University of Texas at Austin

3:00-3:30 Mary Girard/B110
Department of Molecular Virology and Microbiology,
Baylor College of Medicine

3:45-5:15 Invited General and Medical Microbiology Sessions

General Presentation
3:45-4:00 Kelli Palmer/BSB B110

General Session/BSB D109
4:05-4:25 Pierson
4:25-4:45 Chrzanowski
4:45-5:05 Ng
5:05-5:25 Lind

Medical Session/ BSB D110
4:05 McLean
4:25 Kim
4:45 Sim
5:05 Kang

6:15-7:30 Awards and Dinner/Texas Sports Hall of Fame

7:30-8:45 ASM Lecture and Keynote Speaker

***Human Papillomavirus Infection in the Adolescent* – Are You In The Know?**

Dr. Janice M. Matthew- Greer, LSU Health Science Center, Shreveport, LA.
Professor of Research in Pediatrics and Director of Diagnostic Virology Laboratories

Janice Matthews-Greer, Ph.D., D(ABMM) began her career as a medical technologist and returned to school to study the immune response to bacteria. After receiving her Ph.D., she taught undergraduates and has won several teaching awards. In July 1994 she left teaching to develop one of the first diagnostic (hospital-based) "PCR" (molecular) laboratories in the country. Within six months she had the facility up and running for patient care and now is the Medical Director for the Diagnostic Virology Laboratory and the Human Papillomavirus Diagnostic Laboratory, both at LSUHSC-S. Her research has focused on HPV, specifically adolescent infections. She will be giving the lecture, HPV Update: Genital HPV in Adolescents.

SCHEDULE

SATURDAY OCTOBER 20, 2012

This Mini Conference on Undergraduate Education (miniCUE) session will give participants an overview of the methods used in "Scientific Teaching". The goal of Scientific Teaching is to make teaching more scientific. We will explore ways that we can do this in our microbiology classes? We will begin by describing the main approaches and theories important to Scientific Teaching and continue with two session that apply these methods.

The first session will demonstrate how to use Case Studies to make learning relevant to a student's life and to show the larger picture of biology to your classroom. Using an example case, the Human Microbiome (written for the textbook, Biology: How Life Works), and related in-class activity, we will walk you through the backward design techniques used by the text and activity authors to ensure this case and activity connects core concepts, adds relevance, and inspires critical thinking.

The second session will demonstrate how online interactive games can be used to effectively teach science. Created through grants from the National Institutes of Health and the National Science Foundation, Web Adventures are thematic interactive games and simulations driven by specific learning objectives aligned to National Science Education Standards. The idea was to create a free online environment that not only allows students to explore science concepts, conduct virtual laboratory investigations, and role-play science careers, but also enables teachers to reinforce standards. Currently, there are six themed Web Adventures that cover infectious diseases, the neuroscience behind substance abuse, the impact of alcohol on body systems, forensic science, clinical trials, and science careers. While the original target audience was middle school students and high school students, Web Adventures offers examples of how games can be crafted to teach science at any level. Results of field tests in middle school and high school classrooms indicate the efficacy of this methodology in achieving specific learning objectives and the viability of this type of teaching tool. See http://webadventures.rice.edu.

We will then have time to meet with each other and textbook representatives before breaking into small groups for our Microbrew Sessions. The following sessions will be available:

7:30-8:30	Breakfast
8:30-10:00	Educational Sessions ***Scientific Teaching*** - Tamarah Adair Case Studies - Shannon Howard and Elaine ***Strategies to Dissect Tuberculosis Pathogenic Mechanisms*** - Kristi Bowling
10:00-10:30	Coffee Break
10:30-11:30	Microbrew

PLENARY

Strategies to Dissect Tuberculosis Pathogenic Mechanisms

Jeff Cirillo
Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center, Bryan, Texas

Identification of genes directly involved in pathogenesis can be challenging, particularly because genes involved essential biosynthetic and metabolic processes almost always also impact virulence without directly being involved in interactions with the host. We have developed a novel strategy, designated random-inducible controlled expression (RICE), to address this challenge and describe several of the genes identified. Once genes involved in pathogenesis are identified, it is critical that rapid methods are employed that can allow insight into their mechanisms of action in vitro, followed by detailed analysis of their specific role in animal models. Choice of the model systems that are used can be complicated by the fact that none of them completely replicate disease in humans, but this does not reduce their value in the field. We describe some of these strategies and outline how they have been applied to evaluate the functional role of genes identified. In particular, we have focused on how M. tuberculosis transitions from other carbon sources to fatty acids during infection, granuloma formation and latency. Since the majority of those individuals infected with M. tuberculosis bacilli develop latent infections, this stage of infection is one of the most important to obtain a better understanding of disease, yet it remains one of the least well understood stages due to difficulty of analysis. These studies can provide evidence for the mechanistic role(s) of some these genes in pathogenesis as well as strategies for their use in development of novel intervention methods to help prevent or treat tuberculosis.

GENERAL MICROBIOLOGY SESSIONS

The Evolution of Multipartite Genomes in Bacteria

Cheramie P. Trahan
Department of Biological Sciences, Sam Houston State University, Huntsville, Texas 77341

The multipartite genome has been discovered in a large number of species within Proteobacteria; however the mechanism for the origin and evolution of multipartite genome structure remained as one of the important questions in Microbiology. Over fifty bacterial species of Proteobacteria have been reported to possess multiple chromosomes. In all of the organisms, the primary chromosome (CI) encodes more essential protein functions necessary for cell survival than the accessory chromosome (CII) does. CII encodes a large variety of COG functions, among which many genes in CII are involved in metabolism, important for the greater capability of the organism to exploit varied environments. In addition, it was found that accessory chromosomes have independently originated multiple times in Proteobacteria.

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Genome analysis revealed that sequences of CI are highly conserved, whereas those of CII from the corresponding species have rapidly diverged. The results also demonstrated that the level of sequence divergence of CII among non-pathogenic bacterial species is significantly higher than that of CI of the corresponding species. However, the difference in sequence divergence of CI and CII among pathogenic bacteria is not significant. The two mechanisms, relaxed selective constraint and horizontal gene transfer (HGT) from other species, could together or separately lead to the rapid divergence of CII sequences. This study demonstrated that the levels of HGT in CI and CII are similar and thus HGT may not be the main mechanism contributing towards the rapid divergence of CII. Although most orthologous genes in Proteobacteria considered in this study are under purifying selection, genes on CII are less constrained than those on CI, which suggests that the level of selective constraint may be one of the driving forces for the rapid evolution of CII.

Microbial Drivers of Plant Invasions: Evidence of a New Plant Invasion Strategy, Microbially Enhanced Competitive Ability (MECA)

Marnie E. Rout [1],[2] (presenting author), Thomas H. Chrzanowski[3], Ragan M. Callaway[2], and William E. Holben[2]
The USDA-ARS[1], Fort Keogh LARRL, Miles CIty, MT 59301, The University of Montana[2], Missoula, MT 59812, The University of Texas at Arlington[3], Arlington, TX 76019

Invasive plants can profoundly alter ecosystem processes. Attributes like increased growth rates, biomass, and phytochemistry production have been documented in many invasive plants. Current theories suggest these attributes are plant-regulated; however, our research shows that bacterial endosymbionts can regulate these traits in the invasive grass Sorghum halepense. Using culture and molecular approaches, this work has demonstrated that S. halepense harbors several bacterial endophytes within its roots and rhizomes. Five bacterial endophytes were isolated and identified using 16S-rRNA gene sequencing. Physiological functions of these bacteria were confirmed with in vitro studies, including N2-fixation, iron siderophore production, phosphate solubility, and production of the plant-growth hormone indole-3-acetic acid (IAA). Long-term field studies documented alterations to several soil biogeochemical cycles across an invasion gradient. Heavily invaded soils had increased plant-available forms of essential macronutrients (N, P, K, Mg) and trace metals (Cu, Fe, Mn, Zn) compared to moderately- and non-invaded soils. Deep 16S rRNA gene sequencing of non-invaded, transitional and highly invaded soils showed significant differences in microbial community structure, with decreases in soil microbial α- and β-diversity as a function of plant invasion. Using a novel antibiotic-based approach, bacterial activities were restricted within the plant to assess bacterial contributions to invasive plant traits. Bacterial endophytes significantly increased invader biomass and altered resource allocation toward rhizomes. Competition experiments showed plants with active endosymbionts inhibited growth of a native prairie grass frequently displaced by the invader; restricting bacterial activity completely removed these effects, and HPLC analysis of growth medium suggests this is due to restricted secondary metabolite production when endophytes are restricted. Endophyte activity increased production of the herbivore-defense compound, dhurrin, in the leaves of S. halepense. When leaves from plants with active bacterial endosymbionts were fed to a generalist insect herbivore (Tricoplusia

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ni), the insect could not grow and experienced high mortality. Inhibition of endophyte activity resulted in a 6-fold decrease in dhurrin, in conjunction with significant increases in insect growth and survival. Collectively, these results suggest that bacterial endosymbionts significantly contribute to S. halepense invasions through their physiological contributions to soil nutrient cycles and by enhancing multiple invasive plant traits.

The role of tellurium resistance genes in the virulence of Bacillus anthracis

S. Elizabeth Franks[1] (presenting author), Celia Ebrahimi[2], Andrew Hollands[2], Cheryl Y. Okumura[2], Raffi Aroian[2], Victor Nizet[2], and Shauna M. McGillivray[1],[2]
Texas Christian University[1], Fort Worth TX
University of California[2], San Diego, La Jolla CA.

Bacillus anthracis, the causative agent of anthrax, must avoid an array of antibacterial defenses by the host during the course of infection. Although anthrax toxin and capsule, located on two large plasmids, play important roles in the pathogenesis of this disease, evidence indicates that chromosomal genes also contribute. We have generated a random chromosomal mutant library of B. anthracis Sterne using a mariner-based transposon mutagenesis system in order to identify novel chromosomal virulence factors. Bacterial mutants were screened for loss of virulence in the invertebrate animal model Caenorhabditis elegans. C. elegans were fed B. anthracis Sterne under conditions that enabled up to 80% of worms to become infected. Bacterial mutants unable to infect C. elegans were selected for and the site of transposon insertion was identified. In vitro assays assessing susceptibility to specific antimicrobial defenses were used to identify potential mechanisms of action of attenuated mutants. The most highly attenuated mutant was found to have a disruption in an operon containing multiple tellurium resistance genes. We discovered this mutant to have increased susceptibility to potassium tellurite as well as to host defenses such as reactive oxygen species and antimicrobial peptides. In addition, the mutant displays attenuated virulence in whole blood and macrophage survival in comparison to wild type. We conclude that the tellurium resistance operon of B. anthracis has a novel role in resistance to critical host defenses.

Identification of Type Four Secretion Substrates that are Essential for Coxiella burnetii Intracellular Replication

Mary. M. Weber (presenting author), Chen Chen, Kristina Rowin, Katja Mertens, Zhao-Qing Luo, and James E. Samuel
Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center, Bryan, Texas

Coxiella burnetii, the etiological agent of acute and chronic Q fever in humans, is an intracellular pathogen that replicates in an acidified parasitophorous vacuole derived from host lysosomes. Central to pathogenesis is a specialized Dot/Icm secretion system that delivers bacterial virulence proteins, termed effector proteins, into the host cell cytosol. Using a bacterial two hybrid and bioinformatics guided approaches we identified over three hundred candidate secretion substrates. To determine which of these candidate substrates was capable of being secreted, each candidate was expressed as a β-lactamase

continued

TEM1 fusion protein. Using L. pneumophila as a surrogate host, we identified ninety substrates that were secreted in a Dot/Icm dependent manner. In order to understand the role of these substrates in C. burnetii pathogenesis, we used several large scale screening approaches aimed at identifying the function and subcellular localization of T4SS substrates in eukaryotic cells. To determine if any of our substrates could interfere with crucial host pathways, each substrate was heterologously expressed in S. cerevisiae. Substrate induction using 2% galactose identified several substrates that interfered with yeast growth, suggesting these proteins target crucial host pathways and may be bona fide effector proteins. Subcellular localization for each substrate was determined by expressing each substrate as a C-terminal fusion to EGFP. Ectopic expression in HeLa cells revealed that C. burnetii T4SS substrates traffic to distinct subcellular sites, including the endoplasmic reticulum, mitochondria, autophagosomes, and nucleus. To determine if any of these T4SS substrates are necessary for intracellular replication, axenically cultured C. burnetii was transformed with pKM225 encoding the Himar1 transposon and transposase. Genomic insertion of the Himar1 transposon was determined by rescue cloning and sequencing. To date we have isolated 18 clonal T4SS substrate mutants. Growth curve analysis in HeLa and J774 cells identified 4 substrates that were severally impaired in intracellular replication and parasitophorous vacuole formation, but exhibited normal growth in axenic culture. Collectively these results indicate that C. burnetii encodes a large repertoire of T4SS substrates that play an integral role in host cell subversion and PV formation and suggests less redundancy than found for the comparative Legionella model.

Molecular Warfare between a Marine Fungus and a Coral Host is Exacerbated by Elevated Temperature.

Joshuah M. Beach-Letendre (presenting author), Laura D. Mydlarz, and Whitney T. Mann

Invertebrate-microbe interactions within coral reef communities are rapidly shifting out of equilibrium throughout the globe. Higher temperatures are believed to be responsible for this destabilization. One specific example is the increasing disease prevalence amount the Caribbean sea fan, Gorgonia ventalina. Aspergillosis is among the most common sea fan diseases and is associated with the pathogenic marine fungus, Aspergillus sydowii. The purpose of this project was to investigate the effect of temperature increase on protease activity of the fungus in relation to this disease. Fungi produce proteases to obtain nutrients from their environment which can include living tissue as in the case of the sea fan. The sea fan can deactivate these proteases through secretion of protease inhibitors. Extracts from both the fungus and sea fan were tested against a fluorescent labeled protein that fluoresces when cleaved by a protease. A rise in temperature was found to correspond to a rise in protease activity of the marine fungus. Protease inhibition was significantly lowered in diseased colonies compared to healthy colonies. These data suggest that a temperature increase not only results in a higher fungal protease level but may hamper the sea fan's ability to neutralize these destructive enzymes. This project demonstrates the impact a rapidly changing climate can have on one of the most diverse ecosystems on the planet.

Bacteria Lot Identification Matrix Program

Jeff Bardwell (presenting author) and Diane Hartman
Baylor University, Department of Biology

Identifying bacterial colony isolates via computer software matrices is a common methodology used for clinical and environmental microbiology studies. The software analyzes probability matrices utilizing biochemical test results to generate identification probability likelihoods for any given taxa. Several commercial and freeware programs are currently available, but only offer limited functionality. We seek to redress perceived weaknesses in existing software by writing a new program using python 2.7 tkinter programming language. This new software module integrates taxonomically current matrices directly into the program, uses cross-platform operating system compatibility, adds search functionality, includes a full range of statistical analyses, allows for site sampling bias, and uses smaller lot id matrices to reduce the number of biochemical tests required to reach a valid identification.

Elucidating the Microbial Retting Community of Hibiscous cannabinus under Differing Conditions

David K. Visi[1] (presenting author) and Michael S. Allen[2]
University of North Texas[1], Denton, TX
University of North Texas Health Science Center[2], Fort Worth, TX

Crops such as kenaf, hemp, and jute have been used for cordage and fiber production since prehistory. To obtain the fibers, harvested plants are soaked in ponds where the endogenous microflora digests the pectins and other heteropolysaccharides, which release the fibers in a process called retting. Renewed interest in "green" substitutes for inorganic fibers and desire for biodegradable composites has led to the re-evaluation of the retting process. Numerous studies have identified cultivable bacteria from retting solutions, but to date, no exploration has been made of the microbial community using next-generation sequencing methods. In this study, we identified the main microbial community members in the "natural" retting process (C2D4) of the fibrous plant Hibiscus cannabinus (kenaf). We also set up a retting process which tested the natural flora on the plant material only (C1D4). We then tested the system after inoculating with a defined consortium of bacteria (E1D4) previously isolated from the environment using enrichments, and ascertained the extent to which the introduced species could establish themselves in the community. Following 4-day incubations, DNA was extracted and subjected to two-stage nested PCR before sequencing the ~110 bp amplicons on an Ion Torrent PGM. The phylum Firmicutes dominated E1D4. The addition of pectinase-degrading isolates Bacillus DP1 and K1 failed to establish themselves in the surface-attached community at day 4; whereas Paenibacillus DP2 made up approximately 17% of the flora. By comparison, C2D4 had 29% Firmicutes and 55% Bacteroidetes at the phylum level. C1D4 had 54% Firmicutes, 35% Proteobacteria, and 8% Bacteroidetes. This study is only the first step in elucidating the retting process, but it provides invaluable information about the microbial interactions during the retting process which will lead to optimizing fiber quality and process efficiency.

Flavonoids, Novel Membrane Active Antimicrobials Against Staphylococcus aureus.

Md. Zahidul Alam[1] (presenting author), Xiaoqian Wu[1], Li Feng[2], Dianqing Sun[2], and Julian G. Hurdle[1]

Department of Biology, University of Texas at Arlington[1], Department of Pharmaceutical Sciences, College of Pharmacy[2], University of Hawai'i at Hilo, HI

Background: The human pathogen S. aureus is a leading cause of persistent infections that are associated with therapeutic failure. In such infections, S. aureus reside in a non-growing metabolically-inactive state and are phenotypically resistant to most antibiotics. Therefore a need exist for novel antibiotics that can eradicate non-growing S. aureus. In this regard, naturally occurring flavonoids have been considered a potential source for new antibiotics, but their antibacterial mechanism of action remains largely underexplored. In this study we sought to examine analogues of flavonoids in terms of their anti-staphylococcal action and potential to kill non-growing cells.

Method: The MICs of analogues Sun-189 and Sun-196 were determined by broth microdilution against S. aureus Newman. Time-kill kinetics was determined against logarithmic and stationary phase cultures at 1x and 4x MIC. Effects on membrane potential were examined by FACS using the probe DiOC2(3). Effects on Protein, DNA and RNA synthesis were assayed in log phase culture by measuring the incorporation of radiolabelled precursors. Mutation frequencies were determined at 1x and 4x of MIC with Vancomycin and Mupirocin as controls.

Results: Sun-189 and Sun-196 demonstrated potent bactericidal activities against S. aureus, killing by nonlytic mechanisms, producing >3 Log reductions in viability in less than 30 min, with no concomitant drop in OD600 against exponentially growing bacteria. They also showed bactericidal killing against stationary phase cells, producing >3 Log reductions within 1 hour at 4x MIC. Sun-189 disrupted the membrane potential within 10 minutes of exposure and caused nonspecific inhibition of several macromolecular biosynthetic processes (DNA, RNA and protein). The mutation frequency of S. aureus to flavonoid was less than 10^{-9}, comparable to vancomycin.

Discussion: These results indicate that flavonoid has excellent in vitro activities against Log phase and stationary phase S. aureus, resulting from disruption of the staphylococcal membrane potential.

Characterization of Chromosomal Origin of Replication in Rhodobacter sphaeroides 2.4.1

Bat-Erdene Myagmarjav (presenting author) and Madhusudan Choudhary
Department of Biological Sciences, Sam Houston State University, Huntsville, TX

Existence of multipartite genomes in prokaryotes is now a well-known and widely accepted paradigm of prokaryotic genome structure. The genome of Rhodobacter sphaeroides consists of two circular chromosomes and five endogenous plasmids. The existence of multipartite genome requires coordination of DNA replication and proper segregation of each chromosome during bacterial cell division. In order

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to identify chromosomal origins of replication in R. sphaeroides, a combination of bioinformatics and molecular approaches was employed. Using Z-curve analysis, three and five putative origin sites were identified on the primary chromosome (CI) and secondary chromosome (CII), respectively. These putative sites have been amplified from R. sphaeroides genome, and cloned into pLO1 plasmids in E. coli. Plasmid, pLO1, acts as suicide vector in R. sphaeroides, and it also contains kanamycin resistance gene, a selectable marker for screening transformants and exconjugants. The recombinant plasmids were then transferred into R. sphaeroides via conjugation. The R. sphaeroides exconjugants were grown in Sistrom minimal medium with kanamycin, and it could successfully grow either by the autonomous replication of the pLO1 plasmids or by performing a single cross-over recombination that resulted in the pLO1 being incorporated into the homologous region of the chromosomes. The autonomous replication of the recombinant suicide vector in R. sphaeroides was the confirmation of the true origin of replication, and it was distinguished from recombination possibility by a simple PCR experiment. These putative origins of replication were also examined using an alternate approach, real time-PCR, which directly quantifies putative replication origins, and allows us to confirm the true origin of replication. This novel method has not been used in identifications of bacterial origins of replication prior to this study.

Application of Response Surface Methodology for the Optimization of Calcium carbonate Production by Sporosarcina pasteurii.

Tugba O. Okyay (presenting author) and Debora F. Rodrigues
Department of Civil and Environmental Engineering, University of Houston, Houston, TX 77204-5003

The major bioessential elements, C, N, P, S, Si, K and Ca, are efficiently recycled within terrestrial and marine ecosystems. In the case of the calcium element, the mechanisms that cause its accumulation and recycling in the environment are various, but not fully understood. Bacteria and algae have been described as playing an important role in the biogeochemical cycle of calcium and as being affected by environmental factors such as urea, calcium and nickel concentrations. In this study, Sporosarcina pasteurii ATCC 11859 was used to investigate the effects of these environmental factors in the production of CaCO3. Response surface methodology (RSM) was employed to determine the experimental design for these environmental factors in order to reduce the number of experiments and allow simultaneous examination of the effects of the different environmental factors. Experiments were done to obtain cell growth kinetics, pH changes, and ammonium and calcium carbonate production. From these results, the RSM predicted that the optimum urea, calcium and nickel concentrations were 42.12 g/l, 6.93 g/l and 0.072 g/l, respectively, for the production of CaCO3. These concentrations were tested experimentally and compared to previous studies that describe the best experimental medium conditions for the production of CaCO3 for S. pasteurii. Under the optimum predicted conditions by the RSM, the experimental results showed that the calcium carbonate precipitation rate was 0.145 h-1 and the maximum urease activity was 3.4 U/ml at 6 h. These results are 2.5 times higher than the ones described in the literature.

Redundant and multi-functional transaminases of lysine and arginine synthesis in Escherichia coli

Piyush B Lal (presenting author), Barbara Schneider, and Larry Reitzer
University of Texas at Dallas, Department of Molecular and Cell Biology, Richardson, TX

The transaminase ArgD catalyzes the fourth step of arginine synthesis and apparently a reaction in diaminopimelate and lysine synthesis. Deletion of argD did not result in a requirement for either arginine or lysine, which suggested redundant enzymes. A triple mutant lacking ArgD, AstC and SerC was a diaminopimelate auxotroph, which indicates that these enzymes synthesize N-succinyl-L,L-diaminopimelate. Overexpression of all 16 transaminases showed that these three enzymes, as well as seven other transaminases, complemented the defect. Overexpression of all transaminase genes indicated that ArgD, AstC, GabT and PuuE catalyzed the formation of N-acetylornithine in arginine synthesis. A quadruple mutant lacking these enzymes was an arginine auxotroph, which confirms the activity assays. The transaminases in lysine and arginine synthesis are not only redundant, but they also catalyze more than one physiologically relevant reaction.

GOLDSCHMIDT AWARD LECTURE

Evolution of Enhanced Psl Exopolysaccharide Production in Chronic Pseudomonas aeruginosa Cystic Fibrosis Isolates

Holly K. Huse (presenting author), Peter A. Jorth, and Marvin Whiteley
University of Texas at Austin, Department of Molecular Genetics and Microbiology

The Gram-negative bacterium Pseudomonas aeruginosa is a common cause of chronic respiratory infections in individuals with the heritable disease cystic fibrosis (CF). These infections can last for decades, resulting in significant morbidity and mortality. Upon establishment, P. aeruginosa infections are difficult to eradicate because the bacterium is highly persistent. This persistence has been partially attributed to P. aeruginosa's ability to form robust biofilms. While in vitro biofilm growth is well characterized, genetic traits that evolve in vivo and contribute to biofilm formation are less understood. Recently we identified 24 P. aeruginosa genes that were differentially expressed in chronic P. aeruginosa CF isolates compared to their isogenic progenitor strains. The goal of this study was to identify the function of these genes with the overlying hypothesis that some of these genes would promote biofilm formation in chronic P. aeruginosa strains. To test this hypothesis, we constructed strains of the laboratory bacterium P. aeruginosa PAO1 that expressed these genes at levels observed in the chronic isolates. One of these genes, phaF (PA5060), results in enhanced biofilm formation when expressed in PAO1. PhaF promotes biofilm formation via up-regulation of Psl, an exopolysaccharide essential for attachment and biofilm maintenance. PhaF regulates Psl post-transcriptionally, and the mechanism of this control is currently under investigation. Finally, we show that Psl production is enhanced in 8 of 10 chronic CF isolates compared to ancestral strains, suggesting that Psl is an important biofilm-promoting factor in vivo.

GOLDSCHMIDT AWARD LECTURE

License to Kill: Bioengineering a bacterium with Antimicrobial Activity via the Exploitation of Toxin-antitoxin Systems and Conjugation

Mary Girard and Christophe Herman
Departments of Molecular Virology & Microbiology and Molecular & Human Genetics, Baylor College of Medicine, Houston, TX

With the rise in antibiotic resistant bacterial infections, we are in need of new antimicrobial methods. One potential alternative is to exploit the natural bacterial toxin-antitoxin (TA) systems. TA systems are bacterial operons which encode a stable toxin and an unstable corresponding antitoxin. Toxin-antitoxin gene modules are always co-expressed to alleviate toxicity as the toxin is detrimental and will kill or inhibit growth of the bacterium when the antitoxin is depleted. The role of TA systems in bacterial physiology remains unclear; one possible role for TA systems is maintenance and proper segregation of large, low-copy plasmids. If errors occur in plasmid segregation during division, the daughter cell which lacks a TA-encoding plasmid will succumb to the toxin. Accordingly, several TA systems have been found on conjugative plasmids, which are transferred through a sex pilus from the donor to the recipient in a process known as conjugation.

This project aims to investigate a novel antimicrobial approach that exploits conjugation and TA systems to engineer a bacterium that delivers toxins by conjugation. This strategy relies on uncoupling toxin/antitoxin gene expression by placing the toxin gene on the conjugative plasmid and the antitoxin gene on the chromosome. Upon transfer of the plasmid, only the toxins will be expressed, killing the recipient bacterium. We aim to determine whether conjugative transfer of a plasmid encoding multiple toxins will kill recipient bacteria and how this toxic effect can be targeted to specific bacterial pathogens. We are initially testing this antimicrobial strategy by performing mating assays between the engineered strain carrying a single toxin and non-pathogenic laboratory strains of E. coli, scoring for cell viability. Preliminary data shows that this method effectively kills recipient E. coli in both planktonic and biofilm states. Next, we will be testing different combinations of toxins to determine the effect of multiple toxins on cell viability. In the future, mating and viability assays will also be performed with a panel of bacterial pathogens. Ultimately, this work may lead to the development of this system for application in biotherapeutics or biocontainment.

GENERAL PRESENTATION

Horizontal Gene Transfer and Antibiotic Resistance in the Enterococci

Kelli L. Palmer
University of Texas at Dallas

The enterococci are Gram-positive fermentative bacteria and opportunistic pathogens that colonize the gastrointestinal (GI) tracts of diverse hosts. Enterococcus faecalis and E. faecium can colonize extraintestinal sites such as the bloodstream, heart, and urinary tract, leading to potentially fatal disease. Not all strains of E. faecalis and E. faecium are equivalent. Certain phylogenetic lineages within these species are especially associated with hospital-acquired infections and with multidrug resistance acquired through horizontal gene transfer - these are called the high risk lineages. The striking enrichment for mobile genetic elements such as plasmids, transposons, and phage in genomes of high risk strains suggests that endogenous genome (self) defense systems are compromised or absent. Clustered, Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas defense provides a type of acquired immunity against phage and plasmids in the related bacterium Streptococcus thermophilus. My research concerns the relationship of the genome defense system CRISPR-Cas with the emergence and persistence of high risk E. faecalis and E. faecium.

GENERAL SESSION

Regulation of Phenazine biosynthesis by Two-component Signal Transduction Systems in Pseudomonas chlororaphis Strain 30-84

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The rhizosphere-colonizing bacterium Pseudomonas chlororaphis 30-84 is an effective biological control of take-all disease of wheat. Phenazines are bacterial secondary metabolites produced by 30-84 that are essential for the strain's ability to inhibit fungal pathogens, form biofilms, and effectively colonize the rhizosphere. In strain 30-84, phenazine biosynthesis is controlled by a complex regulatory network that includes GacS/GacA, a two-component signal transduction system that is highly conserved among Gram negative bacteria and globally activates the production of many secondary metabolites; PhzI/PhzR, the phenazine quorum sensing system; and other regulatory genes. Previously, we showed that phenazine production also is negatively regulated by RpeA, a putative sensor kinase of a two-component signal transduction system. Recently, we identified the cognate response regulator RpeB. Quantitative real-time PCR revealed that the expression of the phenazine biosynthetic genes as well as the phenazine

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regulatory genes pip (phenazine inducing protein) and phzR were significantly reduced in an rpeB mutant compared to the wild type. However, as expected based on the observed phenotype, expression of these genes was higher in the repA mutant than in the wild type. These results demonstrate that in contrast to RpeA, RpeB positively regulates phenazine biosynthesis. Complementation assays showed that expression of pip in trans rescued phenazine production in the rpeB mutant; whereas expression of rpeB in trans did not restore phenazine production in pip or phzR mutants. These results indicate that RpeA and RpeB differentially regulate phenazine production and act upstream of Pip and PhzR in the phenazine regulatory network. The differential regulatory functions for RpeA and RpeB also affected the capacity of strain 30-84 to inhibit fungal pathogens. Based on these results, we proposed a model illustrating our current understanding of the integration of multiple regulatory genes in the control phenazine biosynthesis in P. chlororaphis 30-84.

The mixotrophic protist Ochromonas danica is an indiscriminant predator whose fitness is influenced by the type of bacteria it preys upon

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Microbial predator-prey interactions are one of the primary trophic interactions linking biogeochemical cycles to ecosystem dynamics. The mixotrophic flagellate Ochromonas danica was used as a model predator to investigate feeding trends when supplied actively growing and non-growing bacteria representing a variety of phylogenetic groups. The rate at which bacteria were ingested and the subsequent growth rate (ecological fitness) of O. danica were determined for each type of prey in each growth state. O. danica preferred bacteria between 0.6 and 1.0 μm3. It was, in general, an indiscriminate predator in that it readily ingested growing and non-growing bacteria spanning a range of phylogenetic groups, with some notable exceptions. Bacilli/Actinobacteria were ingested at significantly lower rates than Alpha- and Gammaproteobacteria. There were no significant differences among rates at which Proteobacteria (Alpha-, Beta- and Gamma-) were ingested. The ecological fitness of O. danica could not be predicted from ingestion rates. Predation on Bacilli/Actinobacteria resulted in growth rates significantly lower than when the flagellate preyed upon Gammaproteobacteria. Betaproteobacteria were readily ingested but always resulted in a significant decline in predator fitness. The data reveal that different types of bacteria have different nutritional value to a consumer. If other flagellates respond similarly, then it would seem that trophic transfer efficiency and nutrient regeneration depend on the diet-breadth of predators.

Recovery of the Soil Microbial Community in a Post-Lignite Surface Mining Chronosequence in Eastern Texas

Justin Park Ho Ng (presenting author), Terry Gentry, and Frank Hons
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Surface mining results in the destruction of the original soil profile characteristics, and alters biological

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properties. Our objective was to determine if the soil microbial community and functionality recovered in a post-lignite surface mining chronosequence in eastern Texas. Eight reclamation sites aged 0 to 40 years were chosen utilizing two reclamation techniques (CP - crosspit spreader, and MO- mixed overburden). An unmined site was used as a control. Soil microbial biomass and mineralization both recovered to premined levels after 10 years. Bacteria and Fungi recovered to premined levels after 20 years. The CP20 site was most closely related to the UM site, but sites 10 years and older were also comparable to the UM site. Relative levels of dominant phyla (Actinobacteria, Acidobacteria and Proteobacteria; comprising >70% of all sequences) returned to premined levels after 10 years, and correlated well with chemical properties and previously defined soil quality indicators, soil microbial biomass and carbon and nitrogen mineralization. Soil functionality did not differ significantly between sites and metabolic diversity peaked at 20 years.

Nitrogen-directed phenotypic plasticity of the toxic cyanobacterium, Cylindrospermopsis raciborskii.

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Cylindrospermopsis raciborskii occurs in distinct morphotypes: wavy, coiled, helical, and straight. It is thought that toxin production differs among morphotypes. In Lake Catemaco, Mexico hepato- and neurotoxins are present in marketed fishes. Both morphotype frequency and inorganic nitrogen concentration co-vary between rainy and dry seasons– an observation leading to the hypothesis that morphotype is determined by inorganic nitrogen concentration. Using lake isolates we tested the nitrogen-morphotype hypothesis. We present experimental evidence that the trichome morphotype and size plasticity potential is expressed by altering nitrogen concentration. With nitrogen-containing media (124 mg N l-1) trichomes were long (225 μm) and wavy. When that N concentration was halved, the trichomes were short straight rods (13 μm). With no nitrogen three morphotypes developed– straight, helical, and coiled with heterocytes on all morphotypes, unlike the Lake where heterocytes occur only on the coiled morphotype. This was initially the case for nitrogen-free cultures, but as the cultures aged, heterocytes developed on all morphotypes. Apparently the heterocyte-morphotype association is environmentally, not genetically, determined because sequencing four genes from lake and cultured samples found no difference among morphotypes.

MEDICAL SESSION

Biofilms - Microbial Life on Surfaces

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When microorganisms are examined in their natural environments, they typically grow as mixed-population, surface-adherent communities referred to as biofilms. Through the work of JW (Bill) Costerton we now know that biofilm growth is very common and that organisms within biofilms are much more resistant to antibiotics and disinfectants than unattached (planktonic) bacteria. Bacterial concentrations within biofilms are high and so population density gene expression (quorum signaling) is important. As part of our work with biofilms, we investigated mixed culture interactions in Pseudomonas aeruginosa and Escherichia coli. Here, we found that E. coli indole production was necessary for its survival in this mixed population. Indole inhibited pyocyanin and other quorum-regulated virulence factors in P. aeruginosa. While indole did affect acylated homoserine lactone-based quorum signaling, we also noted that another quorum regulated molecule (Pseudomonas quinolone signal (PQS)) was also affected. We also observed that indole affected quorum-regulated pigmentation in two other gram negative bacteria, Pseudomonas aureofaciens and Chromobacterium violaceum. Overall, these studies suggest that many complex metabolic and signal interactions occur in surface-adherent biofilm communities.

Peptidoglycan-Tertiary Structures of Staphylococcus aureus and FemA Mutant by Solid-State NMR

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The role of peptidoglycan (PG) inter-bridge lengths on the PG-tertiary structure in intact whole-cells of Staphylococcus aureus and its femA-deletion mutant were investigated. The PG inter-bridge structure in S. aureus is a pentaglycine, whereas in femA-deletion mutant is a monoglycine. The PG-tessera structures of S. aureus and its femA-deletion mutant were characterized by rotational-echo double resonance (REDOR) NMR. REDOR NMR measured the heteronuclear dipolar coupling constants between the isotope labels incorporated into the PG, hence internuclear distances. Isotope labels D-[1-13C]Ala and L-[15N]Ala were incorporated into the PG of intact whole-cells by growing the cells in the presence of an alanine-racemase inhibitor alaphosphin. The 13C-15N distances between the 13C of D-[1-13C]Ala in one PG stem to the L-[15N]Ala of the nearest neighboring stem for both S. aureus and its femA-deletion mutant determined by 13C{15N} and 15N{13C} REDOR were 6 and 5 Å, respectively. The PG-tessera structures were further elucidated by complexing LCTA-1110 (p-fluorophenyl-piperazinamide eremomycin) to the D-[1-13C]Ala and L-[15N]Ala labeled whole-cells. LCTA-1110 is a semisynthetic glycopeptide antibiotic with a fluorine (19F) positioned at the carboxyl terminus, and binds to the PG in cell wall. LCTA-11110 functioned as a molecular probe to measure the distances from

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the 19F of PG-bound LCTA-1110 to the 15N of L-[15N]Ala of drug-bound PG-stem, and to the 13C of D-[1-13C]Ala of the nearest PG-stem. The REDOR distance measurements of S. aureus indicated that two PG-stems cross-linked by a pentaglycycl bridge in cell wall must share the same (or parallel) orientation. This structural constraint provided a model PG-tertiary structure where PG glycan chains are tightly packed together with repeating pseudo-tessera structure in S. aureus. For femA-deletion mutant, a hybrid PG architecture model consisting of both parallel and perpendicular PG-stem cross-links is proposed.

Identification of Culex Complex Species Using SNP Markers Based on High Resolution Melting Analysis

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Culex pipiens complex mosquitoes are primary vectors for diseases such as West Nile encephalitis, Eastern equine encephalitis, many arboviruses, as well as lymphatic filariases. Despite sharing physiological resemblances, each Culex complex mosquito has unique behavioral and reproductive traits that give need for a proper method of identification among the member species. Culex quinquefasciatus, the southern house mosquito, is found in tropical climates, is anautogous (requires blood meals), is stenogamous (needs open spaces to reproduce), and nondiapausing. In contrast the Culex pipiens form pipiens, or the northern house mosquito, is found in temperate climates, and while it is also anautogenous, it is eurygamous, capable of overwintering diapause, and prefers avian blood. Finally, the below ground Culex pipiens form molestus is autogenous, stenogamous (able to mate in confined spaces), nondiapausing, and prefers mammalian bloods. Unfortunately, morphometric methods of distinguishing member species within this complex have failed to yield consistent results, giving rise to a need for molecular methods of identification. In this study we propose a novel identification method using high-resolution melting analysis by examining single nucleotide polymorphisms in the acetylcholinesterase-2 (ace-2) locus. Our method provides a high resolution for species determination among the three Culex complex mosquitoes.

Response of Carbon and Nitrogen cycle functional genes to the prolonged no-till practice and crop rotation in a framing system experiment in North Carolina

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Two major modifications for sustainable agriculture are no-tillage practices and improved crop rotations. Both have been shown to improve soil structure and quality in supporting crop growth and yield. Soil microbes are the mediators of biogeochemical cycles for major nutrients such as C and N. However

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our knowledge of microbial responses to no-tillage and crop rotation is limited to the physiology and structure of microbial communities at a rather coarse resolution. Microarray technology targeting ~47,000 functional genes, GeoChip 3.0, was used to investigate the microbial response in detail, especially for functional constituents in nutrient cycles. A long-term experiment was designed to contrast the impact of conventional tillage (CT), no tillage (NT) and non-managed systems called successional (SC) fields. Soil properties showed significant responses to both no tillage ($R = 0.684$) and crop rotations ($R = 0.523$). The response of microbial community structure was significant to no tillage ($R = 0.063$) and crop rotations ($R = 0.102$) as well. The percentage of functional genes recovered was lowest in SC field, but it was not significantly lower than NT and CT fields. The responses in functional gene diversity (H'), richness and relative abundance were not significant to treatments, but the responses of nitrification and N fixation functional genes in diversity and richness were significant ($P < 0.05$). Functional genes related to three biogenic greenhouse gases did not show significant change to both no tillage and crop rotation. Although microbial community structure was significant to both treatments, the response was stronger to crop rotation, and the treatments were more responsive to the mean state than variance of microbial community structures. Multivariate correlation analysis identified similar sets of soil chemical and physical properties, and they were most significantly associated with NT and CT field functional genes respectively. Together the responses in nutrient cycle functional genes to no-tillage practice and crop rotations were apparent in the structure of microbial communities, but not in diversity and abundance.

ASM LECTURE AND KEYNOTE SPEAKER

Human Papillomavirus Infection in the Adolescent – *Are You In The Know*?

Janice Matthews-Greer, Ph.D., D(ABMM)

Data collected over the past decade by numerous investigators clearly indicate Human papillomavirus (HPV) infection is highly prevalent among adolescents. This is a consequence of both behavioral and biological factors such as anatomic differences between the immature and mature cervix. HPV infection may lead to genital warts, cervical cell changes and, if persistent, to cervical cancer. Beginning our investigation in 2003, we collected cytology and/or histology results, incident data of high (cancer)-risk HPV (hr-HPV) genotypes and their distribution among girls in our population of adolescents receiving cervical cytology screening. We continue to monitor outcome data after infection with specific genotypes in our cohort. Our objectives were to better understand the epidemiology of HPV in adolescents and to describe anticipated changes in the prevalence of hr types due to widespread use of the HPV vaccine containing HPV16/18.

Methods: Until September 2006, HPV DNA testing was performed on all females, including adolescents, undergoing Pap smears that utilized liquid Pap media (ThinPrepR from Hologics) who either (1) had atypical cells of undetermined significance (ASCUS) cytology or (2) if ordered by a physician without reference to specific cytology result. Laboratory records were tabulated by hand. HPV DNA screening was performed on remnant liquid specimens using the Hybrid Capture 2 (HC-2) assay (Qiagen) that detects 13 hr-HPV genotypes, but does not distinguish between them. Genotyping was performed using a nested multiplex PCR with endpoint read by Luminex (Multiplex Genotyping HPV Assay, Qiagen). This assay delineates 2 low(cancer)-risk (lr)-HPV and 17 hr-HPV types. Prealiquots (taken before cytology testing) were removed from liquid Pap smear vials collected from girls between the ages of 10 and 20 from September 2005 to March 2008. Later, those with corresponding cytology results found to be ASCUS were screened for hr-HPV by HC-2 using remnant Pap liquid retrieved from cytology. Genotyping was performed on the prealiquots corresponding to those specimens. Outcome follow-up was done manually.

Results: We found 24% (534/2202) atypical cytology results among girls 11-19 years old compared to 9% (1555/16893) of adult women (p=0.0001). Of those girls with atypical cytology, 85% tested positive for hr-HPV, significantly higher than any other age group, including women aged 20-24 (p=0.003). Of the genotyping cohort, prealiquots were available for only 77 of 121 HC-2-positive adolescent specimens. Multiple genotypes were found in 46% of girls, 4 of whom each had 4 genotypes. Vaccine types were found in less than half (43%) of the girls. The most common HPV genotypes detected were HPV 16 (20%), HPV 52 (12%) and HPV 31 (9%). Only 3% harbored lr-HPV types 6 and/or 11. Repeat cytology and/or HPV screening outcome data was available for 45 girls who fell into 2 groups: girls with improved cytology/virology (n=26) and girls with progression or no improvement (n=19). Comparisons between

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the 2 groups yielded no significant differences in HPV genotypes, multiplicity or types. However, the mean age of the improved group (16.8 years) was significantly lower than girls who progressed (18.0 yrs) (p=0.001). Conclusions: HPV-mediated cervical abnormalities in younger female adolescents are significantly more transient than those of older girls. HPV 16/18 are known to cause 70% of cervical cancers, albeit only 18% of the infections in this young population were due exclusively to those types. Mixed infections comprised almost half of the total cohort with a non-significant trend toward mixed infections in progressors (67%) as compared to improvers (46%). We are continuing to monitor these patients. However, since new guidelines recommend against screening patients for cervical cancer in this age group, it is unlikely that we will do additional testing to enlarge the study cohort.

GRADUATE POSTERS

1. Fluorocytometric Analysis of Membrane Potential in Clostridium Difficile

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Background: C. difficile is a gram-positive obligate anaerobe that is the leading cause of health care associated diarrhea, especially in elderly patients.Typically, C. difficile infections relapse and there are few available treatment options. Recently we proposed that disruption of the bacterial membrane function may provide an approach to rapidly alleviate the burden of CDI, as membrane-active molecules display rapid bactericidal activities involving disruption of the membrane potential and energy depletion. For aerobic bacteria, effective fluorocytometric methods are developed to evaluate effects on the bacterial membrane-potential. Presently, no such method has been reported for C. difficile. As part of our ongoing efforts to discover anti-difficile membrane-active agents, we sought to optimize the standard fluorocytometric membrane-potential assays to confirm the on-target activities of emerging molecules.

Method. Firstly, the bactericidal activities of selected compounds (e.g. reutericyclin-867 and CCCP) were evaluated against C. difficile CD630 by time kill studies. For method development: C. difficile CD630 was cultured in crimped sealed serum bottles in BHITY containing oxygen scavengers (sodium thioglycolate, L-cystine, and NaHCO3); using a hypodermic needle for delivery, the fluorometric probe DiOC2(3) was added and cells incubated for 30 min; compounds were then added and samples withdrawn at various times. Using BD™ LSR II, changes in the membrane potential were assayed based on detection of the red (488-620nm) to green (488-530nm) fluorescence shift in the population. Results. Stationary phase CD630 were rapidly killed by membrane-active agents with concomitant depletion of ATP in a concentration-dependent manner. Disruption of the membrane-potential was confirmed from ratiometric shifts from green to red, which was most prominent for CCCP at 30 min. Discussion. These results indicate that the membrane-potential of C. difficile may be important for its survival. Like other organisms, the clostridial membrane-potential appears to be amendable to assaying with DiOC2(3). Further method refinement is ongoing, including comparison with other known membrane-potential fluorometric probes.

2. Ciclopirox Blocks Growth of Multidrug-Resistant Escherichia coli, Klebsiella pneumoniae, and Acinetobacter baumannii Clinical Isolates

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Multidrug-resistant (MDR) bacterial infections kill ~100,000 Americans annually. We have worked to develop new compounds that are effective against these MDR gram-negative pathogens by derivatizing

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the natural antibiotic, fosmidomycin. We found that the most potent antibiotic derivative was structurally similar to the FDA-approved antifungal drug, ciclopirox. Interestingly, in 30 years of clinical use, no ciclopirox fungal resistance has developed, which could also reduced the likelihood of bacteria developing ciclopirox resistance. Based on our work and published ciclopirox data, we hypothesized that ciclopirox uniquely inhibits growth of MDR gram-negative pathogens.

We measured ciclopirox minimum inhibitory concentrations (MICs) using Clinical Laboratory Standards Institute microbroth dilution method in clinical isolates chosen based upon antibiotic resistance profiles ranging from being completely susceptible to nearly pan-drug resistant. Ciclopirox MICs were 5-15 ¼g/ml for Escherichia coli (n=48), Klebsiella pneumoniae (n=7), and Acinetobacter baumannii (n=8), regardless of how many antibiotics they resisted.

To characterize how ciclopirox affects bacterial growth, we performed a screen using the E. coli ASKA library to find genes that when overexpressed, allowed growth at otherwise restrictive ciclopirox concentrations. The only gene identified was galE. Purified GalE enzymatic function was measured in the presence of ciclopirox; however, no inhibition was found. Because galE functions as part of the galactose salvage pathway, we have hypothesized and shown that bacteria were more sensitive to ciclopirox when galactose was the sole carbon source. This pathway is also a source of nucleotide-charged sugars used to build lipopolysaccharides in gram-negative bacteria. Additional preliminary data analyzing membrane sugar composition by polyacrylamide gel electrophoresis suggests that ciclopirox alters lipopolysaccharide formation in E. coli.

Because it inhibits MDR gram-negative bacteria, ciclopirox may be an excellent candidate antimicrobial agent to treat MDR infections and deriviatize other antibiotics. These data also demonstrate a previously unknown function of ciclopirox affecting the galactose salvage pathway.

3. Pseudomonas aeruginosa Disrupts Tight Junctions Via an EGFR-dependent MAPK Mechanism.

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Pseudomonas aeruginosa frequently infects patients with cystic fibrosis and immunocompromised individuals. One of the first steps in pulmonary injury is the loss of epithelium integrity; which is followed by inflammation and edema. Previous reports from our lab indicate that P. aeruginosa elastase (PE) activates host cells' signal transduction pathways that, in part, contribute to tight junction disruption. The exact mechanism of epithelial disruption, however, is not yet fully understood. We sought to evaluate whether PE-induced activation of epidermal growth factor receptor (EGFR) could modify TJ organization and epithelial barrier integrity, and to characterize the signaling mechanism involved. An in vitro model of human pulmonary epithelial cell (Calu-3) grown on tissue culture inserts was utilized. The confluent monolayers were subsequently treated with P. aeruginosa elastase (2 U/ml, 1hr). Specific inhibitors of EGFR and MAPK (AG1478 and UO126, respectively) were used to assess the

role of targeted signal transduction pathways in PE-induced TJ disruption. Western blotting indicated the activation level of the pathways intermediates and TJ proteins' expression. Changes in transepithelial electrical resistance (TEER) in the presence and absence of PE and aforementioned inhibitors across the confluent cell monolayers were measured using an epithelial voltmeter (Millicell-ERS). The TEER readings showed a significant increase (27.6 ± 4%,).

4. IbeA of Adherent-invasive Escherichia coli (AIEC) Mediates Invasion of Intestinal Epithelial Cells

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Department of Microbiology and Immunology[1], Department of Pathology[2], University of Texas Medical Branch[2], Galveston, TX

Adherent-invasive Escherichia coli (AIEC) is a novel E. coli pathotype under extensive investigation, not just because of its association with the intestine, but due to its link with Crohn's disease (CD) lesions. It is still not well defined whether AIEC can contribute to the onset of CD pathology; however, AIEC displays characteristics that allow the organism to multiply and persist in the ileum of CD patients. Two hallmark features of the AIEC pathogenic phenotype are the invasive capability of the intestinal epithelium and the promotion of an inflammatory state and; therefore, these areas require further study. We hypothesized that IbeA, a putative invasin which gene is found in the genome of AIEC, plays a functional role in the bacterial invasive properties. We have constructed a non-polar ibeA deletion mutant (ΔibeA) and studied whether it has an effect on AIEC adhesion and invasion of Caco-2 intestinal cells. We observed a significant reduction in invasion (P=0.005), while adhesion was not affected. Further, the mutant strain was complemented with the inducible plasmid pRJC01 (plasmid containing the ibeA gene under an arabinose inducible promoter) and found that upon induction, the invasive capabilities were regained. Additionally we transformed the plasmid pRJC01 in a non-invasive E. coli strain to evaluate whether this strain gain invasive properties with the gene alone, or other genes found in the ibe locus were also required. Our results show that IbeA plays a relevant role in the invasion process of AIEC, which could be the initial step of perpetuating itself on the intestinal epithelium and contributing to the immune deregulation observed in the gut of Crohn's disease patients. As part of the future directions, we are testing the role of IbeA invasin in the pathogenesis of AIEC using a murine model, where the colonization of the gut of the wild type and the ΔibeA mutant strains will be monitored using in vivo bioluminescence imaging technology to track bacterial persistence and colonization site in the intestine.

5. Facilitating Effective Expression and Purification of CEA to Enable Studies of an Enhanced Salmonella Tumor Targeting Vehicle for Cancer Therapy

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Background: Studies of microbial pathogenesis and microbe based drug delivery systems often demand evaluation of the interactions between the microbe and the mammalian cell surface as a point of first contact. In developing an anti-cancer Salmonella VNP20009 vehicle displaying antibodies specific for the tumor marker carcinoembryonic antigen (CEA) we required purified protein for labeling studies. Due to the complexities of this macromolecule, it is difficult to produce in large amounts and is prohibitively expensive. We have therefore designed a simple system to produce recombinant CEA in human embryonic kidney (HEK 293T) cells based on a systematic evaluation of post-transcriptional regulatory elements (PTRE) and secretion signals.

Methods: A human codon optimized CEA gene with a polyhistidine sequence replacing the transmembrane anchor and C-terminal domain was inserted into vector pcDNA to give an indication of basal transient expression levels following polyethylenimine (PEI) transfection into HEK293T cells. The CEA gene was then inserted into derivatives bearing various PTRE combinations to determine the optimal production system by analysis of supernatants using Luminex and western blot. The highest producing system was modified by replacement of the natural CEA secretion signal with five heterologous signals and re-examined for yield. Scale up production was in 50 mL spinner flasks of serum free ExCell medium which allowed purification by IMAC and gel filtration followed by titration alongside commercial human CEA.

Results: The CMV intron A and cis-repression sequence appeared to be the best PTRE for soluble CEA expression in the supernatant, though a large amount of protein was still detected within the cell lysate. Using the human optimized Gaussia luciferase signal sequence appeared to produce even more CEA in the supernatant. Highly pure protein as judged by SDS-PAGE analysis could be generated that titrated equivalent to commercial grade material by the Luminex assay. \nConclusions: A step-wise analysis of the signals that can potentially increase the expression levels of complex mammalian cell surface proteins has enabled us to generate several milligrams of soluble CEA that will enable us to pursue studies of Salmonella enhanced for tumor targeting.

6. Regulation of Expression of the M143 Promoter of Mouse Cytomegalovirus

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Human cytomegalovirus (HCMV) is a ubiquitous herpes virus commonly infecting up to 100% of adults.

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Immuno-competent individuals rarely have symptoms. However, HCMV is a major cause of mortality and morbidity in immuno-compromised individuals, such as people with AIDs, cancer, and tissue transplants. HCMV is also one of the major infectious causes of birth defects. Mouse cytomegalovirus (MCMV) is used as a model for HCMV infection, since HCMV is species restricted and infects only humans. MCMV and HCMV share similarities in pathogenesis and structural organization. Hence MCMV is useful to study the function and regulation of conserved genes. Our studies address one of these conserved genes, named m143, which is an essential gene for the virus. The m143 protein participates in the inhibition of the shutdown of protein synthesis mediated by an innate antiviral defense pathway. The same essential function is mediated by homologous proteins in HCMV. The objective of this research is to look at the regulation of the m143 promoter. Sequential deletion mutant analysis of the m143 promoter was done in the context of a secreted embryonic alkaline phosphatase (SEAP) reporter plasmid. Under the conditions used, viral factors are required for the activation of the m143 promoter. The results indicated the presence of at least one repressor binding domain and three activation binding domains in this promoter. Two important viral transcriptional regulators that are expressed rapidly after infection are splice variants (IE1/IE3). The results from co-transfections with a plasmid expressing both indicated that IE1and/orIE3 are sufficient to mediate activation from this promoter. In order to determine which one was involved, the reporter plasmids were co-transfected with a plasmid expressing only IE3. IE3 alone weakly activated this promoter, showing that IE1 also is involved. Comparison of the co-transfection results with infections indicated that other viral factors are involved in the repression of this promoter. Discovery of how the virus mediates the repression of this essential viral gene's promoter could lead to a new ways of controlling CMV.

7. Pangenomic Analysis of Genetic Variants in gyrA, recG, ligB, and mutM Associated with Fluoroquinolone Resistance

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Background: Antibiotic resistance is a major worldwide problem. To search for novel genetic alterations associated with fluoroquinolone resistance, we used next generation sequencing and comparative genomics to identify differences between E. coli clinical isolates that were resistant to fluoroquinolones and those that were not. Non-synonomous, genic SNPs were called relative to the fluoroquinolone-susceptible strain DH10B. Novel SNPs occurred in all (n=146) fluoroquinolone-resistant isolates and fell within the recG, ligB, and mutM genes. The well-known gyrAS83L allele also occurred in all. Our goal was determine how pervasive these variants were across the bacterial pangenome and to understand their contribution to antibiotic resistance. \nMethods: Amino acid sequences across the bacterial pangenome were compared to the sequences from the fluoroquinolone-susceptible strain DH10B using Geneious®. Minimum inhibitory concentrations (MICs) of fluoroquinolone antibiotics were measured using Clinical Laboratory Standards Institute microbroth dilution and E-Test® strips.

Results and Conclusions: Whether from the clinic or the environment, isolates across the family enterobacteriaceae overwhelmingly (89% of 167 in GenBank®) contained the amino acid changes

continued

associated with the SNPs found in fluoroquinolone-resistant E. coli. Nine of these isolates also contain the fluoroquinolone-resistant variant in GyrA; isolates only possess the resistant variant of GyrA if the other three variants are present. 4% of isolates in GenBank® have the recG ligB SNPs (n = 2) or the ligB mutM SNPs (n = 8). The remaining 7% contain none of the resistance-associated variants, and include all E. coli strains typically used in the laboratory. These data demonstrate that the workhorse laboratory E. coli strains do not necessarily reflect the bacterial pangenome. To test whether these pervasive variants in recG, ligB, and mutM affect fluoroquinolone resistance directly, we measured MICs in the Keio knockout strains ΔrecG, ΔligB, and ΔmutM. All three deletion mutants had decreased ciprofloxacin and levofloxacin MICs compared to the isogenic parent strain. This result indicates that variants of recG, ligB, and mutM contribute to flurooquinolone resistance.

8. Role of ClpXP Protease on Bacillus anthracis Resistance to Cell Wall-Acting Antimicrobials

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ClpXP is an intracellular protease consisting of two proteins, ClpP, the proteolytic core, and ClpX, a regulatory ATPase, which regulates the life span of multiple bacterial proteins such as transcriptional regulators, rate-limiting enzymes and damaged proteins. ClpXP is conserved across many bacterial species and is often associated with cellular stresses and has been been implicated in the virulence of several pathogens. We recently demonstrated that the loss of ClpX is critical for the pathogenesis of Bacillus anthracis, a Gram-positive bacterium that is the causative agent of anthrax, and results in an increased susceptibility to cell wall-acting antimicrobial agents, including penicillin, daptomycin and cathelicidin antimicrobial peptides. In order to gain a better understanding of how the loss of ClpX leads to an increase in antimicrobial agent susceptibility, we have examined genes involved in regulating the cell wall and cell wall characteristics that have been previously shown in other pathogens to influence antibiotic resistance. Using a genetic knock-out of ClpX in B. anthracis Sterne (ΔclpX), we collected bacterial RNA and compared the genetic expression of genes in parental and ΔclpX B. anthracis known to regulate antibiotic resistance. We also assessed the ability of the bacteria to withstand autolytic stress using Triton X-100 in order to look at innate cell wall activity. Lastly, we physically examined the bacterial cell wall characteristics between parental and ΔclpX B. anthracis using transmission electron microscopy. We found that there is no significant difference in expression of genes related in autolytic activity in ΔclpX as compared to the parental strain. There was also no difference in autolytic activity as measured by Triton X-100 autolysis assays. We have found there is a slight, yet significant, difference in cell wall thickness between ΔclpX and the parental strain. Interestingly, using the TEM, we have seen abnormal division in the ΔclpX bacteria when grown in RPMI, a mammalian cell culture medium, but not in BHI. Loss of ClpX results in aberrant regulation of genes or proteins related to cell wall synthesis or cell division. This may relate to an increase in antimicrobial susceptibility possibly through flaws in cell wall composition. Studies are currently underway to address this.

9. Pseudomonas aeruginosa Chemotaxis towards Serum in a Thermally Injured Mouse Model of Infection

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Thermal injuries represent a very unique type of physical trauma that, in addition to an inherent increased risk of infection due to the loss of the primary skin barrier, are highly associated with the development of bacterial sepsis. Pseudomonas aeruginosa is a gram-negative, opportunistic pathogen that is exceptionally well adapted at establishing systemic infections in thermally injured patients. It has been reported that the mortality rates associated with gram-negative sepsis are as high as 61% with P. aeruginosa consistently being associated with the highest mortality rate among all causes of bacteremic infection. P. aeruginosa utilizes a unique strategy to initiate systemic infections by forming biofilms around the damaged vasculature within burn wounds. This phenomenon, also referred to as perivascular cuffing, acts as a direct portal for P. aeruginosa's entry into the bloodstream. Our hypothesis is that some component of blood, leaking from the damaged vasculature in burn wounds, acts as a chemoattractant for P. aeruginosa, which then preferentially migrates towards the damaged vasculature and establishes this perivascular cuffing. To test this hypothesis, we employed directional motility assays and have demonstrated that P. aeruginosa has a predilection to chemotax towards mouse serum. Furthermore, this chemotaxis was hampered when serum was stored at -20 degrees Celcius for a period greater than 4 weeks, heated-treated at
80 degrees Celcius, or fractionated based on various molecular weight cut-offs. These results suggest that some component of serum acts as a P. aeruginosa chemoattractant and may help explain why P. aeruginosa is able to locate and surround leaky vasculature within thermally insulted tissue resulting in bacteremia.

10. Monitoring Active Phage Infections on Clinically-Relevant Mature Escherichia coli Biofilms using a lux Chemiluminescence Marker

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The presence of antibiotic-resistant bacterial infections, especially chronic wound infections, continues to be a major problem plaguing hospitals worldwide. Up to 85% of chronic bacterial infections involve a biofilm. Biofilms are made up of communities of bacteria that have affixed themselves to a surface and/or each other and are embedded within a self-produced matrix of extracellular polymeric substance (EPS). Biofilm cells are approximately 1000 times more resistant to antibiotics than are their planktonic counterparts. One potential method of treatment against these antibiotic-resistant biofilm infections is the use of bacterial viruses or phage therapy, using biofilm-specific bacteriophages to lyse and destroy targeted bacteria. Phage therapy has been found to be much more effective when a mixture of phages (a phage cocktail) is added, due to genetic recombination effects between the various phages. Mature

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48-hour lux-transformed bioluminescent E. coli biofilms were infected with various combinations of specific phages and screened using bioluminescence detection equipment for effects on population size and metabolic activity in our experimental model.

11. Characterization of a Novel IncFIB Virulence Plasmid in Salmonella enterica serovar Typhimurium

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Salmonella enterica has become an increasing problem as a pathogen for humans as well as commercial poultry farms. With the acquisition of a IncFIB virulence plasmid, Salmonella enterica serovar Kentucky demonstrated increased virulence similar to that found in avian pathogenic Escherichia coli (APEC) which carries the same plasmid. Virulence genes, such as iss, tsh, iroN, iutA, estA, estsB, and cvaC, associated with the IncFIB plasmid were located on various Salmonella enterica serovar Typhimurium strains. The genes were confirmed to exist on an IncFIB plasmid by conjugation assay and isolated by PCR from the transconjugate. Expression of the virulence genes were confirmed performing phenotypic assays. Acquisition of the IncFIB plasmid by S. Typhimurium is a concern due to the virulence genes associated with the plasmid and the ability to spread by conjugation.

12. The Small Molecule Inhibitor, 4-hydroxyacetophenone, Alters Flagellar Dominance in Chlamydomonas reinhardtii

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Recent studies indicate that every cell in the human body possesses at least one cilium during its lifetime. Recent discoveries of diseases associated with ciliary assembly and/or function, more commonly referred to as ciliopathies, have increased the importance of understanding how these organelles operate. The single celled, photosynthetic, green alga, Chlamydomonas reinhardtii, has long been a model organism for ciliary motility studies, and wild-type (CC-125) cells exhibit positive phototaxis. Here, we examine the effects of the small molecule inhibitor, 4-hydroxyacetophenone, on Chlamydomonas reinhardtii motility. Our data indicates that 4-hydroxyacetophenone alters flagellar dominance in Chlamydomonas cells, and causes them to undergo negative phototaxis. Further, in an effort to localize the target of 4-hydroxyacetophenone, a reactivation of cell models assay was performed both with and without 4-hydroxyacetophenone. Additionally, cells were incubated with 4-hydroxyacetophenone followed by the addition of 0.003% H2O2 to examine the effect of 4-hydroxyacetophenone on the redox poise of the cell. Results from the reactivation of cell models assay indicate there is a significant difference in swimming speed and percent motility in cells reactivated with and without 4-hydroxyacetophenone. This suggests 4-hydroxyacetophenone is most likely targeting an axonemal protein. Additionally, results from the H2O2 study show 4-hydroxyacetophenone either does not affect redox poise of the cell or the redox poise of the cell pathway is more powerful than the pathway in which 4-hydroxyacetophenone alters. Also,

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preliminary data of 4-hydroxyacetophenone affinity chromatography performed on matrix + membrane and axonemal extracts indicates a weak or low affinity of the target protein for 4-hydroxyacetophenone.

13. A Biochemical Analysis of the Quorum Sensing-Like Compounds Secreted by Chlamydomonas reinhardtii that Mimic and Interfere with the Quorum Sensing Pathway of Pseudomonas aeruginosa.

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In a previous study conducted by Teplitski et al., (2003) they discovered that the eukaryotic green alga C. reinhardtii, secretes compounds that mimic and interfere with bacterial quorum sensing (QS). More specifically, the compounds secreted by C. reinhardtii showed stimulatory effects in the LuxR pathway of V. fischeri, the CviR pathway of C. violaceum, and the LasR pathway from P. aeruginosa, and inhibitory effects of the same pathways, but only when in the presence of the cognate acyl-homoserine lactone (AHL). Originally a soft agar overlay containing E. coli reporter strains was used to detect the activity of the compounds via luminescence. For our study an E. coli reporter strain was still used, however a ß-galactosidase assay was performed to confirm activity. Our experiments have shown that the compounds secreted by C. reinhardtii share physical properties that are similar to bacterial AHL's, which include hydrophobicity characteristics as determined by the results of TLC plating, solubility properties as determined by their easy extraction into organic solvents, and degradation properties as determined by the lack of results when assayed via GC-MS. While the physical properties are easy to understand, much still needs to be learned about the chemical properties of the compounds. Focusing on the compounds that mimic and interfere with the LasR pathway, experiments that further describe the physical and chemical characteristics of the compounds secreted by C. reinhardtii will be performed. Once the compounds are fractionated and purified further via TLC plating and using a C-18 reverse phase HPLC column, active purified fractions will then be analyzed via GC-MS and 1H-NMR to determine structural characteristics, allowing for a more complete description of these elusive compounds.

14. Post Transcriptional Regulation of Two Essential Murine Cytomegalovirus Genes.

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Human cytomegalovirus (HCMV) infection is prevalent but usually causes no problem in humans with a sound immune system. However HCMV is life threatening for immunocompromised people such as those with AIDS, organ transplants, or neonates. HCMV only infects humans, limiting the kinds of studies which can be done. Mouse cytomegalovirus (MCMV) is a good model to study function and regulation of conserved genes due to similarities in sequence and pathology. Two such conserved genes m142 and m143 in mouse cytomegalovirus are necessary for viral infection. Protein products shut down protein synthesis mediated by protein kinase R. The same function is mediated by the products

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of homologous genes in human cytomegalovirus. The m142 and m143 mRNAs are detected by 1hr post infection whereas protein is barely detected by 3hr post infection. In contrast to IE1 and IE3 mRNA which are also detected by 1hr post infection but protein is readily detected by 2hr post infection. This discrepancy between the transcription and in protein expression suggests that these m142 and m143 mRNAs are post transcriptionally regulated. I am investigating two possible mechanisms, stability and nuclear cytoplasmic export. Stability of m142 and m143 mRNAs was studied at 1hr and 4hr post infection. Levels of mRNA were evaluated by RTqPCR. They were compared with IE1 and IE3 which does not shows discrepancy. Levels were normalized with PABP transcript which is a cellular mRNA and does not change. Comparison of m142 and m143 transcripts to IE1 and IE3 was done. These preliminary data supports my first hypothesis. My overall hypothesis are there post transcriptional regulation of mRNA going on and my two sub hypothesis are m142 and m143 mRNAs are rapidly degraded or because they are unspliced mRNAs and are not completely exported out of the nucleus. Nuclear cytoplasmic localization studies were complicated by the fact that in our methodology resulted all tested mRNA was altered in localization.

15. Exploring the Molecular Interactions of llama Single Domain Antibodies with Marburgvirus Nucleoprotein Using Rapid Antibody Pairing

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Background: Llama-derived single domain antibodies (sdAbs) have high solubility and stability, and their small size can enable them to bind epitopes that may not necessarily be available to classical immunoglobulins. We have isolated several sdAbs specific for the nucleoproteins (NP) of Marburgvirus and Ebolavirus using in vitro antibody selections on live virus preparations at BSL-4. To advance these sdAbs as diagnostics we would like to understand the molecular interaction between antibody and antigen, and use this information to improve the sensitivity of virus detection. Our goal here is to develop a simple yet effective cell free system that will identify antibody contact residues and gain of function mutants within the context of a monoclonal antigen capture (sandwich) assay where a single sdAb clone is both captor and tracer.

Methods: Hapten-mediated rapid pairing of parental recombinant antibodies was demonstrated from small-scale osmotic shockates of E. coli that express sdAbs in the periplasmic compartment from a tac promoter based system. Site directed mutagenesis was used to alter a key contact residue in CDR3, and mutants were analyzed for their ability to bind recombinant NP using the shockate derived pairing system. Concomitantly, T7 promoter-based cytosolic sdAb expression was evaluated for compatibility with rapid antibody pairing with a view to reproduce the mutant analysis in a system one step away from a cell-free protein expression extract.

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Results: Mutants could be easily screened for antigen capture ability using rapid antibody pairing, though an extra N-terminal sdAb tag would offer a convenient way to confirm presence of captor sdAbs. SdAbs could be produced in the cytosol and bead beating used to release the proteins for successful analysis via antibody pairing, though again an extra tag would be a bonus. We are currently evaluating cell-free expression extracts to synthesize our sdAb from linear T7 promoter expression cassettes derived by PCR amplification.

Conclusion: Deciphering the interactions between Marburgvirus NP and sdAbs will be made easier and more cost-effective with this cell-free adapted rapid antibody pairing and will provide a molecular roadmap towards better diagnostics for virus detection.

16. The Cloacal Microbiomes of Antibiotic-treated versus Untreated Chicks of the Critically Endangered Attwater's Prairie-chicken

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Attwater's prairie-chickens (Tympanuchus cupido attwateri) are critically endangered with a wild population of nearly 100 individuals. The wild population has minimal recruitment and is reliant on the annual reintroduction of ~200 captive-raised individuals. The captive breeding program was established in 1992 with a founding population of 17 individuals and is now in its 19th generation. Survival of released birds to 120 days post release ranges from 22-76%, and the annual survival is 10-43% of the birds that are released. For the Attwater's prairie-chicken recovery program to succeed, post-release survival rates must be improved. We proposed that one factor limiting post-release survival to be altered gastrointestinal microbiota as a result of antibiotic administration. The normal gastrointestinal microbiota has been linked to healthy immune function.

Cloacal samples were collected from individuals at the Fossil Rim Wildlife Center just prior to release. Individual DNA samples were pooled based on closeness in age and whether or not the individuals had received antibiotics as chicks. Equimolar amounts of DNA were combined from 7 individuals that received no antibiotics and similarly from 5 individuals that received antibiotics as chicks. The pooled samples were PCR amplified using universal 16S rRNA primers, 27F and 1492R. PCR products were purified and then further amplified targeting the V5 region. Samples were prepared on the Ion Torrent One Touch and ES prior to sequencing on the PGM as per the manufacturer's instructions.

Analysis of the cloacal microbiota in antibiotic-treated versus untreated Attwater's prairie-chickens revealed a significant difference in bacterial populations. The untreated pool community was dominated by Class Bacilli, including members of the Orders of Bacilliales and Lactobacilliales, while the treated pool was dominated by strains of the Class Clostridia. These findings are consistent with reports of post-antibiotic-use rise in Clostridia in the human gut (e.g. C. difficile) and may have important implications for captive breeding programs and survival of reintroduced endangered species.

17. Electron Beam Irradiation Technology Used to Develop Fresh Fruit for the Neutropenic Diet: E-beam Reduces the Microbial Load on the Fruit while Preserving Sensory Attributes and Vital Nutrients

Bianca Smith (presenting author), Katherine G. McElhany, Rachel Acevedo, Rosemary Walzem, and Suresh D. Pillai
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Immuno-compromised patients are at a high risk for contracting opportunistic microbial infections. This is especially true of neutropenic patients, those with white blood cell counts of < 1500 neutrophils/μL of blood. Because of their immuno-compromised state, these patients are urged to follow a diet that reduces the potential of exposure to microbial populations. This regimen can be difficult for children to adhere to because they are unable to eat foods such as fresh fruit due to the risk of microbial exposure. E-Beam irradiation is an FDA approved food processing technique that has many applications. The objectives of this study are to evaluate the use of E-Beam irradiation at currently FDA-approved doses (< 1 kGy) to determine whether microbial numbers on fresh fruits can be reduced.

Four fruits (strawberries, red grapes, cherry tomatoes, and watermelon) were chosen because of their proven nutritional and anticancer properties. Avocado was included in the study as a high-fat fruit to determine whether E-Beam would cause undesirable oxidation in this fruit. Aerobic plate count methods are being employed to determine the microbial loads of E-Beam irradiated and non-irradiated samples. Additionally the effect of Modified Atmosphere packaging (MAP) on the reduction of microbial populations is also being investigated. A previously identified bacterial plate count benchmark of < 500 CFU/ gram will be used to determine the applicability of the E-Beam treatment. Subsequent to the microbiological evaluation of the fruits sensory properties of the E-Beam treated fruits will be performed to delineate the optimal conditions for the E-Beam treatment of fruits for pediatric cancer patients. A consumer study as well as instrumentation measuring color, texture and moisture content will be used to compare the treated fruit to the control. A nutrient analysis will also be carried out to determine whether there was a significant loss in vital nutrients when the fruit were irradiated. Ascorbic acid, phenolic acids, sugar content, pH and Total soluble solids will be tested between the two treatments.

18. Insulin treatment enhances Pseudomonas aeruginosa biofilm formation in the diabetic wound environment

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Diabetes affects 25.8 million people in the U.S., or 8.3% of the population, and these numbers are even higher in developing countries. Diabetic patients are more susceptible to the development of chronic wounds with debilitating bacterial infections than non-diabetics. Previously, we developed an in-vivo chronic wound, diabetic mouse model to examine the ability of the opportunistic pathogen, P. aeruginosa, to cause biofilm-associated infections. Unexpectedly, more biofilm was observed in the wounds of insulin-treated diabetic mice, which correlated with higher antibiotic tolerance. Previous studies have suggested that insulin treatment for burn wounds decreases the acute, planktonic growth of P. aeruginosa in favor of a chronic, biofilm mode of growth. Also, insulin treatment is well known to exert anti-inflammatory effects on both diabetic and non-diabetic human cells. Taken together, these previous reports and our in vivo data raised the possibility that insulin treatment either directly or indirectly affects P. aeruginosa, resulting in more biofilm formation. Thus, we hypothesize that insulin treatment either directly affects the bacteria in the wound, promoting growth/biofilm formation, or indirectly affects the immune response in a manner that favors infection and/or biofilm formation (e.g. delaying initial inflammation). We observed that while insulin did not directly affect P. aeruginosa's growth, it did promote biofilm formation and increased gentamicin tolerance in vitro and these effects were negated when cells were exposed to heat-denatured insulin. Utilizing microarrays only small changes in gene expression were observed after P. aeruginosa was exposed to active insulin in vitro, the mRNA levels of an L-lactate permease (lldP) and several heat shock proteins were increased above twofold. Utilizing immunohistochemistry and immunofluorescence we observed significantly less macrophages in the wounds of insulin-treated diabetic mice. Overall these data suggest that systemic insulin treatment both directly and indirectly favors P. aeruginosa biofilm growth in the diabetic environment.

UNDERGRADUATE POSTERS

19. Staphylococcus aureus: an absolute victim of Pseudomonas aeruginosa's opportunism.

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Infection sites are usually colonized by different gram-positive and gram-negative pathogens. Within the infection site, pathogenic bacteria exist in specific structures termed biofilms, which protect the bacteria from the host response and antibiotics. Within the biofilm, bacteria may support each other's growth or compete with each other for the limited nutrients. Such competition may completely eliminate some pathogens from the biofilm. The gram-positive microorganism Staphylococcus aureus and the gram-negative microorganism Pseudomonas aeruginosa are commonly isolated from diverse infection sites. In this study, we utilized the three-dimensional biofilm model to examine the nature of the interaction between P. aeruginosa and S. aureus. The biofilm medium used consisted of nutrient broth (Bacto-peptone), collagen, and agar. To visualize the biofilms by confocal laser scanning microscopy, we used the P. aeruginosa strain PAO1/pMP7065 and the S. aureus strain AH133, which carry the genes for the red and green fluorescent proteins, respectively. When individually inoculated and at 48 hours post-inoculation, AH133 produced a mature well-developed biofilm but PAO1/pMP7065 failed to develop any biofilm. However, quantitative analysis revealed that the planktonic cells of the two strains reached a comparable level of growth in the biofilm medium indicating that the medium does not inhibit the planktonic growth of PAO1. In the presence of serum, when we inoculated PAO1/pMP7065 onto a partially developed AH133 biofilm, PAO1/pMP7065 developed a mature biofilm at 24 hours post inoculation. At 72 hours post inoculation, the mixed species biofilm predominantly consisted of PAO1/pMP7065. To determine if AH133 produces an extracellular factor that supports the development of PAO1/pMP7065 biofilm, we added AH133 supernatant to the biofilm medium used to culture PAO1/pMP7065. Despite that, PAO1/pMP7065 failed to develop a biofilm. These results suggest that, in our biofilm model, P. aeruginosa totally depends on its direct contact with S. aureus to develop its own biofilm; yet, as P. aeruginosa grow, it gradually eliminates S. aureus from the mixed species biofilm.

20. Metagenomic Analysis of Body Decomposition

Jordan Baker (presenting author), Dr. Aaron M. Lynne, and Dr. Sibyl Bucheli
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While alive, organisms ranging from small mosquitos to a human serve as unique ecosystems that play host to a wide range of other organisms such as parasitic worms, viruses, and enteric bacteria. While there are many studies, such as the Human Microbiome Project, to better understand the microbial diversity in a living human, the diversity of microbes involved in the stages of body decomposition is not well studied. It is generally understood that there are five general stages for decomposition: fresh, bloat,

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decay, post-decay, and skeletal remains. How long a corpse stays in each phase is dependent on biotic and abiotic factors such as bacteria, scavengers, insects, climate, and weather. In this study, the microbiome of a corpse will be catalogued by sampling bacteria in various locations in the body at two determined conditions; pre and post bloat. Bacterial specimens present in the decomposing corpse will be examined using methods consistent with those that have been developed for the NIH Human Microbiome Project using 16S rRNA genes on the 454-Titanium platform. Once the organisms characteristic of each stage of decomposition are identified, the metabolic pathways and growth requirements of key organisms can be dissected to help us understand how they impact decomposition.

21. Expression and Purification of Crotamine in BL21 Escherichia coli Cells, and its Effects on Murine Myoblasts and Melanoma Cells Using Microcell Culture

Aaron Beach[1], Jonda Halcomb[1], Morgan McLarty[2], Lauren Balderas[2], and Dr. Elda E. Sánchez[2]

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Escherichia coli cells containing expression plasmids were selectively grown at optimized conditions in LB medium containing 50 µg of ampicillin. Recombinant crotamine (r-CRO), containing His6-tag, was purified utilizing Talon® Metal Affinity Resins. Crude venom from the snake was fractionated using High Performance Liquid Chromatography (HPLC) and yielded a positively charged molecule in fraction one, which was shown to have neurotoxic and myotoxic effects and activity of temporary hind leg paralysis in female BALB/c mice. This basic polypeptide belongs to a group of myotoxins that have natural cell penetrating peptides, and is approximately 68 amino acids long. Purified r-CRO was tested for cytotoxic effects per isoform, as well as native CRO. Microcell culture assays were performed on C2C12 murine myoblasts and SK-MEL-28 carcinoma cells using seven native and seven recombinant isoforms of crotamine (CRO). The seven isoforms of crotamine were identified through a cDNA library constructed from the venom gland of a Southern Pacific rattlesnake (Crotalus oreganus helleri). Each crotamine isoform fragment was amplified using Novagen pET32-b containing an N-terminal His6-tag by PCR. After restriction enzyme digestion the PCR products were transformed into BL21 competent cells (Invitrogen). Crotamine has been suggested to have analgesic activity, which could be beneficial to the pharmaceutical industry in the treatment of chronic pain and various muscular disorders.

22. Interactions of Pseudomonas aeruginosa and Staphylococcus aureus in an in Vitro Wound Model

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Chronic wound infections have a profound effect on a large patient population and cost billions of dollars

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in medical costs annually in the US. The microbial populations¬¬¬ of these infections are typically polymicrobial, biofilm-associated, and display increased tolerance to antimicrobials, but despite the prevalence and severity of wound infections, microbial interactions in this environment have been significantly understudied. Two of the most prominent and notoriously resistant bacterial species found together in wounds are Pseudomonas aeruginosa (PA) and Staphylococcus aureus (SA). However, few studies have examined the interactions of these two species in the context of wound infections. Recently, a simple method to grow polymicrobial biofilms in vitro was established, which uses a chopped-meat and blood plasma-based media. This model reliably supports the growth of polymicrobial biofilms, which accurately reflect the composition of human wound infections. We used this model to examine the interspecies relationship between SA and PA in an in vitro 'wound-like' environment. We examined the physical dynamics of the two species in this wound biofilm model and compared the antimicrobial tolerance of planktonic cells to that of biofilm-associated cells. Our results demonstrated that: 1. When PA and SA were grown in planktonic cell co-cultures, PA quickly killed SA; however, when they were grown in our wound biofilm model, PA did not kill SA. 2. SA alone was readily able to form a host-matrix-associated wound biofilm. 3. PA was not able to form its own biofilm, but was able to colonize the biofilm made by SA, when the two were grown together. 4. Imaging analysis of the two species within the biofilm demonstrated many single-species microcolonies that were often located in close proximity to each other. 5. Strikingly, while no SA planktonic cells survived gentamicin treatment, over half of SA cells in wound biofilms remained viable after treatment, and 91% of SA cells from SA/PA dual-species biofilms remained viable after treatment. Taken together, these data indicate that within the wound environment SA and PA may have synergistic interactions that allow them to coexist within a host-matrix-associated biofilm, which results in the benefit of increased antimicrobial tolerance for both species.

23. The Effects of Oxygen Culture Levels on the Adaptive Response of Two-Dimensional (2D) Mono Layer and Three-Dimensional (3D) Epithelial Micro-Cell Culture Systems

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The Adaptive Response (AR) is a protective phenomenon in which cells exposed to a low-dose of ionizing radiation are transiently protected against a variety of effects from subsequent higher exposures. In the present studies human mammary epithelial cells (MCF10A) were grown in cell culture systems to investigate the effect of a low-dose X-ray exposure prior to a high challenging dose. Micronuclei (MN) were scored as markers of induced DNA damage, which appear as extra nuclear bodies that are formed from un- or mis-rejoined double strand breaks (dsb). Scoring the formation of MN in bi-nucleated cells can be used as a biomarker for genomic instability. MCF10A cells were grown in 2D mono-layers and 3D cell culture systems at atmospheric (20% O2) or physiological (3% O2) oxygen levels. MCF10A cells were exposed to a low adaptive dose of 10 cGy and challenged 4 hours later with a higher dose of 2 Gy of X-rays. Mono-layer cell cultures were used to perform proliferation tests, and it was discovered that MCF10A cells grow slightly better under a more physiological oxygen concentration of 3%. Cells growing in the 3D gel matrices showed cell morphology more like intact tissue by forming acinar structures.

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Morphology studies in 3D showed that the branched tubular growth patterns occurred more often in 3% oxygen which better mimics in vivo conditions. Future experiments will help us gain a better understanding of the effects of chronic low-dose radiation exposure to human cells by using a beta-ray emitter.

24. Identification of Frataxin Homolog in Rhodobacter sphaeroides

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Friedreich's ataxia is a rare recessive neurodegenerative disease resulting from insufficient expression of mitochondrial protein called Frataxin. Frataxin protein is coded by the FXN gene located on chromosome 9. In the normal version of the gene, a sequence of DNA (GAA) is repeated between 7 and 22 times in the first intron, while in the mutant gene GAA repeats occur more than 100 times resulting in the neurodegenerative symptoms. Previous studies have shown that Frataxin-homologs are present in many distantly related evolutionary lineages, including fungi, eubacteria, and archaea. Previously, it has been hypothesized that due to mutation in Frataxin gene, there is accumulation of iron in the mitochondria which reacts with oxygen and forms free radicals destroying the cells of the central nervous system. To ensure that Frataxin plays a role in iron metabolism, we chose Rhodobacter sphaeroides as the model organism. A similarity search was performed using blastp. Frataxin homolog (RSP_1650) was identified in R. sphaeroides genome, which codes for hypothetical protein. Protein sequences of Frataxin homologs were aligned using a multiple protein alignment algorithm, ClustalW. Seven putative iron-binding sites were found among Frataxin homologs of R. sphaeroides, Saccharomyces cerevisiae, and Homo sapiens. This result corroborated the idea that these homologs have very similar or identical function and play an important role in iron metabolism. A further genetic and molecular analysis will be employed in R. sphaeroides in which frataxin gene homolog (RSP_1650) will be knocked-out experimentally and its effects on iron metabolism will be investigated.

25. Assessment of the Feasibility of Exposure of Song Birds to Potentially Aflatoxin Contaminated Grain by Observation of Ground and Perch Feeders

Kristina Lardner [2] (presenting author), Jonda Halcomb[2], J. Robert Hatherill[2], Alan M. Fedynich[1], Shad D. Nelson[1], Scott E. Henke[1]
Caesar Kleberg Wildlife Research Institute Texas A & M University[1], Kingsville, Texas and Del Mar College[2], Corpus Christi, Texas

Aspergillus flavus is a eukaryotic fungus that can infect the seeds of agronomical important crops that include corn, peanut, and cottonseed. Growth of A. flavus on a food source often produces aflatoxin, a carcinogenic and highly toxic secondary fungal metabolite that belongs to a class of compounds known as mycotoxins. When ingested by animals it can result in poisoning causing aflatoxicosis. In free ranging migratory birds one of the two types of mycotoxin poisoning that has been documented is aflatoxicosis. Birds are more susceptible than mammals and young birds are more susceptible than adult birds.

continued

Information exists on the practice of supplemental feeding or baiting and the potential of exposure to aflatoxin contaminated feed of wild game animals such as deer, wild turkey and bobwhite, but few studies are available for other non targeted avian species that these practices also attract.

The study area utilized was the Tio and Janell Kleberg Wildlife Research Park located in Kingsville Texas. Two methods of supplemental feeding were applied at five feeding stations; Ground and elevated perching platform feeders using a 50/50 blend of milo and corn as supplemental feed. Feeding stations were observed one day per five day week 2 times per day. Bird preference of feeder type was recorded.

Of the twenty one species counted over a period of twenty days, sparrow abundance comprised 51% of the community studied. White-winged doves comprised 20%, the other 19 species comprised the remaining 29%. The top two in species abundance are birds that are gregarious in nature. The preference of these birds to forage as a flock has the possibility to expose a greater number of birds to potentially aflatoxin contaminated grains at one time. Further study is recommended to assess if this behavior is detrimental to these species.

26. The Microbiome of the Fish Gambusia affinis: Highly Selective

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How the bacteria in microbiomes on animals function and interact as ecological systems is a question of importance to both basic microbiological and biomedical applied science. Gambusia affinis, a small freshwater fish was used as a model organism to study microbiomes. When the bacteria extracted from the fish skin were cultured, it was surprising to find that they appeared to be exclusively gram negative, since Gram positive species are not lacking in the aquatic environment. Colony number on EMB, SS, and MacConkey's was equivalent to that on NA, while MSA and CSA obtained no colonies. Lack of culturable Gram positive bacteria indicate either selectivity of the slime layer, or lack of ability to isolate them on media and certainly required further investigation. 16S profile sequencing was performed on samples from four fish. Gram positive genera accounted for 6% in one fish, and less than 1% in the others. Five Gram negative genera accounted for more than 40% of the microbiome sequences. The skin of Gambusia affinis is highly selective, and dominated by limited set of commensals. The selectivity was explored experimentally, by exposed fish to high dose challenges of representative bacteria in the water column. Micrococcus luteus (Gram positive non-pathogen) was unsuccessful at colonization after a 48 hr exposure. Escherichia coli (Gram negative non-pathogen) revealed initial short-term colonization, followed by loss of the invasive species over four days. The mechanism of this exclusion is under investigation.

27. Discovery of Novel Iron Acquisition Genes in Bacillus anthracis

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Bacillus anthracis is a gram-positive, rod-shaped and spore forming bacterium. It is considered a Category A agent by the Centers for Disease Control and Prevention due to its threat to society. B. anthracis is the causative agent of the deadly disease Anthrax. In order for bacteria to cause disease, they must evade many defenses of the host immune system. Our objective is to discover novel virulence factors that B. anthracis uses to overcome the host's ability to fight infection. Iron is an important nutrient for growth and proliferation of the bacteria, which pathogens must obtain from their host environment such as from hemoglobin found in red blood cells. It is known that B. anthracis uses two types of siderophores proteins, IsdX1 and IsdX2 a family of the ISDB proteins, to strip iron from the hemoglobin. We believe that additional novel proteins enable B. anthracis to acquire iron from hemoglobin that may work in conjunction with ISDB proteins. We used a transposon library that has random mutations within the genes of the Sterne strain of B. anthracis and screened for mutants that are unable to acquire iron from hemoglobin. We have identified 13 mutants out of 960 that are unable to acquire iron from hemoglobin when compared to the wild type B. anthracis. We have identified several of the disrupted genes using a linker PCR method and are following up on the remaining mutants. We hope our results will provide insight as to what genes are necessary for iron is acquired from hemoglobin. This will further our understanding of B. anthracis pathogenesis may open up new targets for pharmacological intervention.

28. Testing Antibody Binding and Specific Bio-Markers in Endothelial Micro Cell Culture Systems.

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This study investigated fluorescent DAPI staining through antibody binding by measuring the fluorescent signal to confirm the presence of endothelial/vascular cells. The isolation of the Stromal Vascular Fraction (SVF) from adipose tissue yields material rich in multipotent cells. These multipotent cells have been used in numerous research studies to repair defects in bone, cartilage, and soft tissues. A key point in moving this work from research bench to the bedside for clinical studies is the ability consistently isolate, store and retrieve the SVF in a viable state. Samples of human SVF were taken out of cryogenic storage and the cellular viability was assessed using a hemocytometer with trypan blue and 6-diamidino-2-phenylindole (DAPI) for viability assays by light and fluorescent microscopy. The SVF then underwent staining by antibodies to help identify specific cell types verifying the SVF was isolated and retrieved. The antibody binding of seven cell type markers: CD31, CD34, CD44, CD45, Siglec, ALCAM and Collagen IV. These antibodies stain endothelial/vascular cells and will bind to specific sites on stromal cells. The extracellular matrix was used to verify that the SVF was successfully recovered from cryopreservation

continued

storage. It was concluded that 44 percent of CD34 stained the endothelial/vascular cells, signifying the presence of the SVF.

29. In-frame Deletion of recA in Rhodobacter sphaeroides 2.4.1

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Rhodobacter sphaeroides 2.4.1 is a purple non-sulfur bacterium belonging to the **α**-3 subdivision of the Proteobacteria, and its genome is comprised of two circular chromosomes and five endogenous plasmids. The recA gene encodes for the RecA enzyme, a DNA-dependent ATPase, which is vital for homologous recombination during the cell cycle. RecA also assists in the mediation of the SOS response by inducing autoproteolysis of the LexA repressor enzyme, and regulates error-prone DNA synthesis that bypasses DNA lesions. To perform the in-frame deletion, two pairs of PCR primers were designed, which produce two PCR products containing corresponding 3' and 5' ends that are complementary to each other. PCR amplification of the regions flanking recA both upstream and downstream is first performed. A recA deletion allele is constructed through the fusion of these complementary sequences using a subsequent round of PCR, which utilizes primers with overhang regions. The fusion product is then cloned into the suicide vector (pLO1), and the recA deletion construction is confirmed through sequencing of the cloned insert. This plasmid contains a kanamycin resistance (Kmr) gene, a selectable marker, which allows for screening of transformants or exconjugants. Kanamycin resistant (Kmr) colonies of R. sphaeroides grown in liquid culture are selected for and plated on LB agar containing 15% sucrose, which selects for a double crossover event that allows for the replacement of the wild type recA gene by the deletion allele. Verification of gene replacement is performed using PCR and subsequently a DNA sequencing method. Since the recA gene and its homologs are conserved across all bacterial species, suppressing homologous recombination in R. sphaeroides through utilization of an in-frame deletion would allow this bacterium to be used as a key biotechnological tool for maintaining cloned heterologous genes. In addition, the recA mutant may be utilized to facilitate studies of the SOS response in this bacterium.

30. Regulation of Expression from the m142 Promoter of Mouce Cytomegalovirus

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Worldwide 60 to 100 % of adults are infected with human cytomegalovirus (HCMV), but it generally causes no problems in people with a healthy immune system. However HCMV causes serious illness or death in immune-compromised people, such as organ transplant recipients or AIDS patients and it is a major infectious cause of birth defects. HCMV can only infect humans, limiting the studies done with this virus to tissue culture. The closely related mouse cytomegalovirus (MCMV) has similarity in both sequence and diseases caused, thus can act as a model to study function and regulation of conserved genes. One such conserved gene in MCMV is m142, which is essential for inhibiting protein kinase R (PKR) mediated shut off of protein synthesis. The m142 protein is a functional homolog of IRS1 and

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TRS1 of HCMV. Our long term goal is to identify regulatory mechanisms for the m142 promoter. This could lead to new therapies. We have created a series of approximately 100 bps sequential deletions within the m142 promoter controlling expression of the secreted alkaline phosphatase (SEAP). Promoter function was quantified by measuring the amount of SEAP produced and transfection efficiency was normalized by qPCR for plasmid DNA. Under the conditions used, viral proteins are essential for detectable activation of the m142 promoter. Deletion analysis showed at least two possible repressor and three activator binding regions. Further experiments were focused on the repressor binding regions because understanding repression mechanisms could lead to developing possible controls. Viral binding and viral structural proteins are not involved in the repression of the m142 promoter. Co-transfection with potent viral activators ie1 and ie3 showed repression pattern similar to viral infection suggesting involvement of cellular repressors. Deletion of a putative Yin-Yang1 (YY1) site in one of the identified regions suggested involvement of cellular factor YY1 in the repression of the m142 promoter.

31. Mycobacterium smegmatis Bacteriophage Gene Annotation and Study of Host Specificity Using Agrobacterium Tumefaciens, Escherichia coli and Saccharomyces cerevisiae as Aternative Hosts.

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Twent- six Bacteriophages isolated from different areas of the Corpus Christi, TX region have been studied to determine host specificity. Gene annotation of phage 'Jsquared' identified a lysine B gene , with the bioinformatics software DNA Master, Phamerator and HHPred. The lysin B genea novel mycolylarabinogalactan esterase, was found to closely resemble similar genes found in Rhizobium radiobacter (formerly Agrobacterium tumefaciens) and many plant species. Bacteriophages are able to infect specific types of bacterial hosts. Once they attach to the bacteria and penetrate through the cell wall, they can begin to replicate themselves often lysing the bacteria. This can be identified by plaque formations. Bacteriophages are the most abundant life form on Earth. Phages use many mechanisms to allow successful infection and can transfer and incorporate DNA between themselves and bacteria. The genes contained in a bacteriophage genome vary between phages and are important in their manipulation of bacteria. Since 'Jsquared' contains a lysine B gene similar to the plant bacterim A. tumefaciens the possibility for phage infection was studied. All twenty six bacteriophages originally isolated with M. smegmatis were plated with A. tumefaciens, E. coli and S.cerevisiae. A. tumefaciens LBA4404 cells were grown on YM plates in a 30°C incubator. E. coli was grown on LB broth plates at 37°C and S. cerevisiae was grown on YPD plates at 30°C incubation. For each of the bacterial or yeast ells grown ,twenty-six areas were marked with a grid and 2ul of phage lysate were introduced into the grid under a sterilized laminar flow hood. Phage buffer was used as a negative control for all plates. Plaque formation outcomes were recorded after 3-5 days to determine if infection occurred. None of the bacteriophages were able to infect any of the plated bacterial or yeast cells. All of the phages used in this experiment have been identified as host specific bacteriophages to M.smegmatis.

32. Fusing DNA Repair Enzymes with iLOV for Fluorescent Protein Expression in E. coli

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Preserving structural integrity of the genetic code is one of the major challenges of cells. Everyday our cells battle against genetic damage that can lead to cancer or premature aging. Defects in DNA repair machinery have devastating effects on human health and quality of life. ATPases/helicases, XPD and XPB, are core components of the Transcription Factor II H (TFIIH) repair complex. These proteins are critical for opening damaged DNA and play an essential role in preventing the formation of mutations. Defects in XPD and XPB during nucleotide excision repair (NER) result in a number of diseases. Although Xeroderma Pigmentosum (XP), which causes extreme sun-mediated skin cancer is the most common consequence of this genetic defect, Cockayne Syndrome (CS) and Trichothiodystrophy (TTD) are two diseases caused that are characterized by stunted growth, neurological deterioration, and premature aging. XPD and XPB must unwind distorted lesions in the DNA, in order to allow other NER proteins to carry out repair. With as little as a single amino change, these diseases have different fates, yet all are due to defects in these helicases. The genes, which code for XPD and XPB, are amplified using template DNA from human constructs and transformed into competent E. coli. After the genes of interest are inserted into the pET28a+_iLOV vector and expressed in E. coli, protein purification can be visualized. We hypothesize that fusing the light, oxygen, or voltage sensing, iLOV fluorescent tag to DNA repair genes will assist in expression, purification, and anaerobic crystallization techniques. Results from organisms such as archeae allow for the comparative analysis of the critical components involved in DNA damage repair pathways. Analyzing XPD and XPB provide a foundation for understanding the disease consequences of mutations in DNA helicases.

33. The Effect of Blue-light Irradiation on Staphylococcus aureus in Different Liquid Media

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Methicillin-resistant Staphylococcus aureus (MRSA) is a type of staphylococcus that is resistant to beta-lactams antibiotics such as methicillin, oxacillin, and the like. MRSA infection represents an increasingly serious public health issue and is a recurrent problem in healthcare facilities. Therefore, investigation for innovative antimicrobial therapies to treat such infections is of great interest. One such treatment, antimicrobial photodynamic therapy (aPDT), uses the combination of a photosensitizer with visible light to produce reactive oxygen species (ROS) that leads to cell death. Previous results from our lab suggested that S. aureus may have an endogenous photosensitizer, since an inhibitory effect was seen on S. aureus growth after exposure to 470 nm blue light alone. However, other data indicate that the inhibitory effect seen on S. aureus may be due to the components in the broth. Our hypothesis is twofold: 1) Broth components influence the inhibitory effect of blue light seen on S. aureus. 2) The combination of a photosensitizer with 470 nm blue light produces reactive oxygen species that lead to cell death.

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In order to test the first part of our hypothesis, we have compared the effect of blue light alone on S. aureus in various broth components and additives. Using two 24 well plates with one placed in a blue light box, and the other in a no light box as a control, we illuminated 1 mL of S. aureus cells diluted to 3 x 103 CFUs/mL in the various broths for 30 minutes. The resulting broths were plated on TSA and colony counts recorded after 24 hours of growth. Our results indicate that the inhibitory effect is seen in Brain Heart Infusion (BHI), but not in Mueller Hinton (MH), PBS, Luria Broth (LB), yeast digest, tryptone, peptone, or a mixture of amino acids. To test the second part of our hypothesis, we added ascorbic acid in BHI alone and repeated the previous protocol. The addition of this known RO scavenger significantly decreased the inhibitory effect of blue light.

34. Comparative Immunity Analysis in Mycobacterium smegmatis and Mycobacteriophage

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The purpose of this research is to expand the library of information related to mycobacteriophages and the relationship between those viruses and the bacteria that are immune to them. This project used Mycobacterium smegmatis as the host bacteria as it is of the same genus of the causative organisms of Tuberculosis and Leprosy but is innocuous to humans. This research used mycobacteriophages isolated from the National Genomics Research Initiative classes at UNT from 2009 and 2010, which are members of a variety of clusters and subclusters. The lysogenic pathway was the focus of the project because it involves the insertion of the mycobacteriophage DNA onto the bacterial chromosome. To isolate bacteria that are immune to certain mycobacteriophages, the bacteria were infected by the phage, and mesas where are a film of growth that occurs on top of a zone of clearing established by mycobacteriophages were allowed to form. A small amount was picked from the mesa and then streaked for isolation. After growing this colony into a large culture batch, immunity assays were performed which involved infecting the lysogen of the phage against the 53 phages isolated at UNT. Some interesting immunity patterns were observed. The lysogen of the mycobacteriophage Adephagia named Adephagia L1 is immune to the phage Lew as well as nine other phages. Lew L1 is immune to Adephagia and the nine phages with the addition of the phage Tootziepop. While their lysogens are immune to each other's phage, they do not share the immunity to Tootziepop. Future research will focus on why immunity is or is not transferred. Although some regulatory sequences called Stoperators that mediate immunity have been sequenced in cluster A phages, there do not seem to be any in the cluster that Adephagia belongs to (cluster K). Many lysogens isolated were immune to only one phage which means they are likely very unique. Information from this project can help aid in the decision on what phage should be sequenced next, as well as provide a means to examine how the phage genome is transferred into the bacteria.

35. Using the Saccharomyces cerevisiae Strain Y2HGold to Characterize the Role of

Arabidopsis thaliana Comparative Gene Identification-58.

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The yeast two-hybrid screening system is a highly advanced technique that allows for the detection and characterization of novel protein-protein interactions (PPIs). Many eukaryotic transcription factors have separable DNA binding and transcriptional activation domains, allowing us to test for PPIs by fusing proteins to each domain which, in turn, activates the transcription of reporter genes when an interaction takes place. In the presence of GAL4-based protein interactions, the Saccharomyces cerevisiae strain, Y2HGold, expresses the four reporter constructs, ADE2, AUR1-C, HIS3, and MEL1, minimizing false positives and background. By utilizing the unique properties of this strain, we are able to perform exceptionally stringent yeast two-hybrid (Y2H) assays when testing for novel PPIs. Our primary goal was to characterize the role of Arabidopsis thaliana Comparative Gene Identification-58 (CGI-58), an alpha/beta hydrolase protein responsible for maintaining triacylglycerol levels in plant cells and a participant in the signaling pathways associated with the stress related hormone, abscisic acid (ABA). To gain insight to the potential role of CGI-58 in the lipid and ABA signaling pathways, an Arabidopsis cDNA expression library was screened using the Y2H system, with CGI-58 as "bait." The bait plasmid was then transformed into the haploid yeast strain Y2HGold, and then transgenic yeast cells were mated with complementary S. cerevisiae cells harboring the Arabidopsis expression library. Our findings using the Y2H system were as follows: (i) the secretion of alpha-galactosidase from transformed Y2HGold cells produced a colorimetric change, indicating that CGI-58 strongly interacts with PXA1, a peroxisomal ATP binding cassette protein known to be important for seed germination and metabolism of lipid hormones; and (ii) CGI-58 may weakly interact with the Pyrabactin Resistance 1 and PYR1-Like proteins involved in the ABA signaling pathway, as indicated by S. cerevisiae growth on lower-level selective media. In summary, the S. cerevisiae strain, Y2HGold, and Y2H system has proved itself an invaluable tool in determining that the CGI-58 protein not only plays a vital role in a variety of lipid signaling pathways but that it may also be involved in the sensing of ABA, which is essential for plant health when exposed to various environmental stresses.

36. Investigating the Role of DSS1 in Homologous Recombination DNA Repair in Escherichia coli and Human Osteosarcoma Cells

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To better understand the effect external sources (i.e. ionizing radiation) have on the DNA break repair process, research is being performed with Escherichia coli and the human osteosarcoma cell line (U2OS-DRGFP). When a mutated DSS1, a DNA repair conjugation protein is expressed in E. coli it was lethal. In contrast when mutated DSS1 was expressed in human osteosarcoma cell lines (U2OS-DRGFP), the cells lived. This suggests that other co-mediator proteins participate in vitro in human cells compared

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to E. coli. From the U2OS-direct repeat green fluorescent protein (DRGFP) parental line 35 sub-clonal isolates were derived. The derivative cell line consisted of, 12 wild type, 11 mutant, and 12 cMyc (DSS1 conjugation protein). Using Western blot similar expression levels were determined and compared among cell lines. Ionizing radiation tests were performed on cell lines to test the effectiveness of ectopic DSS1 in Homologous Recombination Repair (HRR) during double strand DNA breaks. The goal of this research is to determine the role DSS1 plays in HRR and its interaction with other recombination mediators.

37. Structural organization and Expression of Flagellar Genes in Rhodobacter sphaeroides 2.4.1

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Rhodobacter sphaeroides 2.4.1 belongs to α-3 subdivision of the Proteobacteria, and it possesses the multipartite genome consisting of two circular chromosomes, primary chromosome (CI) and secondary chromosome (CII). R. sphaeroides displays a wide range of metabolic diversity and also occupies a wide range of ecological niches from freshwater to soil. In bacteria, it has been reported that ~40 genes are required for structure, assembly and regulation of flagellum formation. R. sphaeroides contains two clusters of flagellar genes on CI, first cluster (between 1,736,242 and 1,951,757 base-pairs) and second cluster (between 3,074,540 and 3,105,787 base-pairs). These two gene clusters are responsible for the formation of two different types of flagella. Polar flagellum is responsible for swimming in the liquid medium, while lateral flagella are important in swarming in a dense medium. First and second clusters encode 38 and 21 flagellar proteins, respectively. More than 60 percent (36 out of 59) of flagellar genes exist as duplicate copies in the genome of R. sphaeroides. This result revealed that majority of these duplicated genes might have originated prior to R. sphaeroides formation, and they represented Out-paralogs. It is hypothesized that one of the flagellar gene clusters may have been resulted from gene duplication or/and acquired through horizontal gene transfer (HGT). In this study, a combination of genome analysis and mRNA expression analysis of all the flagellar genes is employed to provide the information about the nature of origin of these gene-pairs in R. sphaeroides.

38. Restriction Enzyme Analysis of the Mycobacteriophage 'Hydrafoxglove'

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Bacteriophages are viruses that infect bacteria. Bacteria often use specific restriction enzymes (REs) to cleave double stranded DNA at specific points into fragments, which are then degraded further by other endonucleases. REs prevent phage infection by effectively destroying the foreign DNA introduced by bacteriophages. Phages without other protection would be expected to modify or eliminate the RE recognition sites from their genomes to avoid RE activity when infecting the host bacteria. DNA modification exists in all types of organisms and varies from species to species. DNA methylation is one

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DNA modification bacteria can undergo and involves the addition of a methyl group to the 5 position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring. The methylation of native DNA acts as a sort of primitive immune system, allowing the bacteria to protect its DNA from digest by the REs and instead degrading bacteriophage DNA. Through evolution, some bacteriophage may methylate its DNA as a mechanism to survive in the host bacteria. During this study, we tested a bacteriophage for the presence of methylated DNA. The bacteriophage 'Hydrafoxglove' was isolated from muddy topsoil in the Texas Gulf Coast area. The genomic DNA was isolated from this phage and later subjected to several restriction enzyme digests. The genomic DNA from 'Hydrafoxglove' resisted digestion by six restriction enzymes BamHI, ClaI, EcoRI, HaeIII, and HindIII, which are known to cut most phage DNA, suggesting the presence of a DNA modification. The phage was also not cut when tested for methylation by using the methylated site-specific restriction enzymes DpnI, DpnII, and McrBC. The phage was further tested for the presence of phosphorothiation by using T5 and T7 exonucleases. The preliminary data suggests that 'Hydrafoxglove' did not have methylated DNA; however further testing is required to determine if phosphorothiation of the DNA has occurred.

38. Presence of Methylmercury Resistance Gene Does Not Correlate with Cefazolin Resistance in Vibrio vulnificus Isolates from South Texas

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Mercury resistant bacteria have adapted to the presence of mercury by evolving enzymatic activity to reduce Hg (II) into Hg (0) (via the merA gene) or by the demethylation of mercury (via the merB gene). Because some mer operons are encoded on transposable elements, it was hypothesized that only naturally selected bacteria would carry the mer gene and that a PCR- based strategy could be used for detection. Further, since preliminary results have revealed a widespread occurrence of the merB Hg resistance gene in sediment and seagrass epiphytic bacteria along the Coastal Bend of Texas and some mercury resistant bacteria share transposable elements with antibiotic resistant operons, it was further hypothesized that the distribution of Hg resistance determinants, specifically merB, may be determined by selection for antibiotic resistance.

V. vulnificus bacteria, a human pathogen found in coastal waters, was assayed by PCR detection of merB in isolates characterized for resistance to 18 antibiotics. Of the 28 isolates from Copano Bay included in this study, 15 showed full or intermediate resistance to Cefazolin, while 13 were susceptible to all of the antibiotics. However, all 28 were found to contain the merB gene. Sequence analysis of merB amplicons from three resistant and one nonresistant isolate produced four identical sequences. Thus, the widespread occurrence of merB did not correlate with resistance to Cefazolin.

39. The Bacterial Flora of Repository Fossils: Sources, Survival and Removal

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Microbes are well known for their ability to degrade a wide range of substances, including rock and bone. Fossils are generally of similar composition as the rock matrix that they are found in, suggesting that microbes known to weather rocks may also affect fossils. Although there is considerable attention and effort applied to the preservation and conservation of fossils for long term storage, research concerning the detection and prevention of microbial growth is lacking in the scientific literature. Given the premise that microbes could damage fossils, our research question focuses on the presence of bacteria on fossils that are stored in repositories. Roughly twenty fossil bones, varying in age, species, and bone type were tested for presence of culturable bacteria. Fossils were swabbed with sterile cotton tip swabs which were streaked onto R2A and nutrient agar plates. Colonies that grew over time were identified using staining and biochemical tests. Additional experimental protocols were also used to determine the state of bacteria found on the fossils. Bacteria that were present on these fossils were extracted into microcentrifuge tubes and were heated to near boiling point to kill vegetative cells, selecting those that produce spores to survive. Initial results from both experiments indicate that microbial counts on stored fossils are relatively low and that the bacteria that are present are predominantly gram-positive, chained bacteria, with evidence that the microbes are in an active, vegetative state on fossils. The persistence of human-derived and soil-derived microbes was also investigated. Human-derived microbes were examined by the addition of concentrated solutions of the ubiquitous human skin bacterium Staphylococcus epidermidis onto decontaminated fossils. Soil-derived microbial population was also tracked over a time by using fresh fossils recovered from the Whiskey Bridge Formation near Bryan, Texas. Human-derived microbes were determined to survive on fossils for relatively short durations, with 98% of S. epidermidis dying off within 24 hours; while soil-derived microbes persisted much longer, with 60% reduction over a time span of 3 months. This work paves the way for examination of potential bacterial degradation/modification of fossils.

40. Identification of the angucyclinone WS5995B monooxygenase and putative biosynthetic gene cluster from Streptomyces acidiscabies 84.104

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Polyketides are a class of microbially-derived natural products which exhibit a diverse range of biological activities. The Gram-positive actinomycete Streptomyces acidiscabies 84.104 produces the antimicrobial aromatic type II angucyclinone polyketides WS5995A, WS5995B and WS5995C. These compounds are structurally simple and consequently most of the enzymatic steps leading to their synthesis can be readily deduced based on comparisons to other known aromatic type II polyketide synthase (PKS) pathways. However, they also undergo an unusual post-cyclization ring cleavage reaction that is not readily assignable to known PKS enzyme activities. In order to understand this step in WS5995

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biosynthesis, we identified the putative WS5995 biosynthetic gene cluster using cosmid screening and genome sequence analysis approaches. Using degenerate ketoacyl synthase probes, type II PKS cosmid clones were identified. Cosmid sequencing resulted in the identification of a monooxygenase gene adjacent to ketoacyl synthase (KS) and chain length factor (CLF) genes. Monooxygenase mutants were generated and found to be defective in the conversion of WS5995B to WS5995C. Analysis of the genome sequence flanking the monooxygenase and KS/CLF genes revealed ORFs with high similarity to genes required for angucyclinone polyketide assembly. Based on these similarities, a likely pathway and gene assignments for enzymatic steps in WS5995 assembly is proposed. In addition, several oxygenase-type ORFs were identified within the gene cluster. It is possible that one or more of these ORFs may play a role in oxidative C-C bond ring cleavage. We are currently investigating this question as the characterization of a novel PKS assembly step may be valuable in the design of novel engineered polyketides with useful activities.