Annual Meeting of the Missouri Valley Branch of the American Society of Microbiology

April 4-6, 2013

Oklahoma State University in Tulsa, OK

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I would like to welcome you to Tulsa and the 2013 Missouri Valley Branch Annual Meeting on behalf of President Julie Shaffer and the local Host Committee. We have planned this year's conference with the hope that you will have a pleasurable and informative visit to our fair city and the Center for Health Sciences and Tulsa campuses of Oklahoma State University. As always, we encourage the active participation of microbiologists at all levels as we exchange fellowship and research findings while working to increase the size and diversity of our Branch. Please do not hesitate to ask any of the host committee members if you need assistance in any way. Welcome to T-town!

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Gerwald Koehler, OSU Center for Health Sciences

Thursday April 4

Time	Activity	Location
7:30 – 9:00 pm	"**If the Yeast Ain't Happy, Ain't Nobody Happy**" Social with Marshall Brewing Company	Holiday Inn –Tulsa City Center 17 West 7th Street

Friday April 5

7:30 – 8:10 am	Registration and Breakfast	OSU-Tulsa North Hall (NH)
8:10 – 8:15 am	Opening remarks	OSU-Tulsa NH150
	Cell and Molecular Biology	
8:15 – 8:30 am	**Analysis of *Gα5* and *RegA* Gene Disruptions on Development in *Dictyostelium discoideum,*** Jamison Miller	OSU-Tulsa NH150
8:30 – 8:45 am	**Role of PKA Overexpression in *Dictyostelium* Chemotaxis, Development, and Cell Differentiation**, Rachel L. Rice	OSU-Tulsa NH150
8:45 – 9:00 am	**Loss of cAMP-specific Phosphodiesterase Rescues Spore Production in *Dictyostelium* G Protein Mutant,** David Schwebs	OSU-Tulsa NH150
9:00 – 9:15 am	**Effector Role of Invasion Plasmid Antigen D (IpaD) of the type III secretion system (T3SS) from *Shigella flexneri*,** Olivia Arizmendi	OSU-Tulsa NH150
9:15 – 9:30 am	**Gelatin-hyaluronic acid scaffolds for intestinal tissue engineering,** Vahid Shabafrooz	OSU-Tulsa NH150
9:30 – 9:45 am	**Tissue Engineering Conductive Scaffolds to Employ Bacteriostatic Effect of Electrical Stimuli,** Aref Shahini	OSU-Tulsa NH150
9:45 – 10:00 am	**Microbial and Molecular Analysis of Morgellons Disease Samples,** Lindsey Allen	OSU-Tulsa NH150
10:00 – 10:15 am	Break	OSU-Tulsa NH
	General Microbiology	
10:15 – 10:30 am	**A Biochemical and Biophysical Characterization of AzoC, the Azoreductase Enzyme of *Clostridium perfringens,*** Jessica Morrison	OSU-Tulsa NH150
10:30 – 10:45 am	**Role of RND Transport Systems in Calcium-Induced Antibiotic Resistance and Virulence in *Pseudomonas aeruginosa,*** Sharmily Khanam	OSU-Tulsa NH150
10:45 – 11:00 am	**Three Functional β-Carbonic Anhydrases in *P. aeruginosa* PAO1. Role in Survival in Ambient Air and Calcification,** Shalaka R. Lotlikar	OSU-Tulsa NH150
11:00 – 11:15 am	**Calcium Homeostasis in *Pseudomonas aeruginosa* Requires Mutliple Transporters,** Manita Guragain	OSU-Tulsa NH150
11:15 – 11:30 am	**Prevalence of Plasmids in *Campylobacter* spp. Isolated From Oklahoma Retail Meats,** Daya Marasini	OSU-Tulsa NH150
11:30 – 11:45 am	**Green Synthesis of Gelatin Based Antibacterial Macroporous Hybrid Scaffolds of Gelatin/Nanosilver/Bioactive Glass for Bone Tissue Engineering,** *Mostafa YazdiMamaghani*	OSU-Tulsa NH150

11:45 am – 1:00 pm	Lunch (Sandwich boxed lunches)	Outside NH150
	Environmental Microbiology	
1:00 – 1:15 pm	**Evaluation of the Phylogenetic Diversity and Community Structure of Members of Candidate Phylum OP8 Using *in silico* Data Mining Approaches,** Ibrahim F. Farag	OSU-Tulsa NH150
1:15 – 1:30 pm	**Genomic Analysis of Hydrocarbon Degradation in *Arhodomonas* sp. strain Seminole.** Sonal Dalvi	OSU-Tulsa NH150
1:30 – 1:45 pm	**Genome Sequence of the Anaerobic Gut Fungi *Orpinomyces* sp. strain C1A,** MB Couger	OSU-Tulsa NH150
1:45 – 2:00 pm	**Cell Envelope Permeability Properties for Nonpolar Compounds in Bacteria Isolated from Waste Water Effluents,** Michelle A. DeGear	OSU-Tulsa NH150
	Medical Microbiology/Immunology	
2:00 – 2:15 pm	**Biophysical characterization for IpaD-IpaB fusion complex from *Shigella flexneri* as a candidate subunit vaccine,** Xiaotong Chen	OSU-Tulsa NH150
2:15 – 2:30 pm	**Biophysical characterization and stabilization of CagL, an antigenic protein from *Helicobater pylori* as a candidate subunit vaccine,** Shyamal Choudhuri	OSU-Tulsa NH150
2:30 - 2:45 pm	Break	OSU-Tulsa NH
2:45 – 3:00 pm	**Evaluating the Antibody Response Following Experimental Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).** Benjamin R. Trible	OSU-Tulsa NH150
3:00 – 3:15 pm	**Protective Efficacy of SPI-1 and SPI-2 Proteins in a Mouse *Salmonella* Model,** Kelly Harrison	OSU-Tulsa NH150
3:15 – 3:30 pm	**Characterizing the Protein-Lipid Interaction of Invasion Plasmid Antigen B (IpaB) from *Shigella flexneri* ,** Philip R. Adam	OSU-Tulsa NH150
3:30 - 3:45 pm	**Identification of probiotic genes of lactobacilli isolated from prairie voles using next generation sequencing,** Simone Bigelow	OSU-Tulsa NH150
3:45 – 4:00 pm	***Desulfovibrio spp.* and Mercury Methylation in the Gastrointestinal Tract of Prairie Voles**, David Supeck	OSU-Tulsa NH150
4:00 – 4:15 pm	**Effect of Monosaccharide Supplementation on Biofilm Formation in Variant Strains of *Burkholderia multivorans,*** Sallie A. Ruskoski	OSU-Tulsa NH150
4:15 – 4:45 pm	Symposium Dr. Donald Rowen "**Use of IMG-ACT and Metacyc for Undergraduate Genomic Analysis Assignments**"	OSU-Tulsa NH150
4:45 – 6:00 pm	Travel to OSU Center for Health Sciences	
6:00 – 8:00 pm	Business meeting, banquet and **ASM Distinguished Lecturer** presentation by **Dr. Nancy Freitag:** "**The *Listeria monocytogenes* Transition from Environmental Bacterium to Human Pathogen**".	OSU – CHS Founders Hall

Saturday April 6

8:00 – 8:30 am	Breakfast	OSU-CHS C.A.M.E. Building Foyer outside D007
	Undergraduate and High School Presentations	
8:30 – 8:45 am	**Characterization of the Putative DnaK-suppressor Protein in the Lyme Disease Spirochete *Borrelia burgdorferi*.** Tia Hadley	OSU-CHS D007
8:45 – 9:00 am	**Comparative In Vitro Antimicrobial Efficacies of Polyphenol Extracts on Non-pathogenic Bacteria and MRSA and MSSA,** J. Gage Holleman	OSU-CHS D007
9:00 – 9:15 am	**A Phylogenetic Analysis of Bacteria in Western Nebraska Alkaline-Saline Lakes Using an Afex Pretreated Cellulose Enrichment,** Kevin Ripp	OSU-CHS D007
9:15 – 9:30 am	**Identification of Siderophore Producing Bacteria from Alkaline-Saline Lakes**, Tyler Lee	OSU-CHS D007
9:30 – 9:45 am	**Characterization of Azoreductase Activity of Whole Cell *Enterococcus faecium* under Aerobic and Anaerobic Conditions,** Keely Redhage	OSU-CHS D007
9:45 – 10:00 am	**Examination of Type III Toxin-Chaperone Interaction by Copurification,** Nicholas Kinkead	OSU-CHS D007
10:00 – 10:15 am	Break	OSU-CHS
10:15 – 10:30 am	**Genetic Variation in the *cmeR* Gene Among *Campylobacter coli* Strains Isolated From Turkey, Swine, and Cattle.** Mohamed K. Fakhr	OSU-CHS D007
10:30 – 10:45 am	**Fungal Diversity of a Cottonwood Root System** Jeff A. Shaw	OSU-CHS D007
10:45 – 11:00 am	**Growth and Glutathione Levels of *Enterococcus faecium* in Response to Quinone Exposure.** Bryan R. Fritz	OSU-CHS D007
11:00 – 11:15 am	**Differential Response of Animal and Environmental Isolates of *Escherichia coli* to Bovine Bile (Ox gal), Deoxycholate, pH, incubation temperature and NaCl,** Jessica M. Robertson	OSU-CHS D007
11:15 – 11:30 am	**Co-Infections of Rocky Mountain Spotted Fever and Ehrlichiosis in American-English Crossbred Fox Hounds – A Case Study,** Laura M. Ruskoski	OSU-CHS D007
11:30 am	Student Awards and Concluding Remarks	OSU-CHS D007

American Society for Microbiology
Distinguished Lecturer Presentation
Dr. Nancy E. Freitag, Ph.D.

The *Listeria monocytogenes* Transition from Environmental Bacterium to Human Pathogen

Many organisms inhabit soil and water, yet a relatively small percentage of these organisms have developed the capacity to cause human disease. *L. monocytogenes*, long used as a model for understanding innate and adaptive immune responses and various aspects of cell biology, also serves as a model for deciphering how a bacterium transitions from life in the soil to life within the cytosol. The Freitag Lab has been working to define how a key transcriptional regulator known as PrfA functions as the molecular switch that balances *L. monocytogenes* life as a saprophyte with life as an intracellular parasite.

Dr. Freitag is a professor in the Department of Microbiology and Immunology at the University of Illinois at Chicago.

Biographical Sketch – Nancy E. Freitag, Ph.D.

Dr. Freitag has been continuously funded for her research on different aspects of *Listeria monocytogenes* pathogenesis for over 15 years. She is internationally recognized for her work on microbe-host interactions, and she has published more than 50 papers and book chapters while also serving as a member of journal editorial boards that include *Infection and Immunity* and *Molecular Microbiology*. Dr. Freitag has been a member or *ad hoc* member on numerous NIH study section panels, and she has served as an *ad hoc* reviewer for international foundations such as the Wellcome Trust. Dr. Freitag has been an invited speaker at more than 80 conferences, seminars, and colloquia, and has participated in a number of Science Career forums organized by various foundations, community colleges, and universities. She maintains an active interest in science education, and has been actively engaged in high school teacher and student science research mentoring programs. In addition, she has served for five years as an ASM judge for the Intel International Science and Engineering Fair.

Cell and Molecular Biology (Convener Dr. Earl Blewett)

Analysis of *Gα5* and *RegA* Gene Disruptions on Development in *Dictyostelium discoideum*.

Jamison Miller*(Undergraduate), David Schwebs, and Jeff Hadwiger
Oklahoma State University, Stillwater, Oklahoma

During the aggregation and multicellular development of *Dictyostelium*, cells communicate with each other via G protein-coupled receptor signaling pathways. Several different Gα subunits are expressed and these contribute to the transduction of specific signaling pathways. Disruption of the Gα5 subunit gene results in delayed development but the subsequent disruption of the phosphodiesterase gene *RegA* accelerated development. Prestalk specific gene expression was analyzed using *lacZ* reporter genes in *gα5*$^-$ and *ga5*$^-$*regA*$^-$ cells. Prestalk gene expression is delayed in *ga5*$^-$ cells but accelerated in *ga5*$^-$*regA*$^-$ cells. This acceleration is the result of intercellular signaling because wild-type or *ga5*$^-$*regA*$^-$ cells can provide intercellular signals in chimeric organisms to accelerate prestalk gene expression in *ga5*$^-$ cells.

Role of PKA Overexpression in *Dictyostelium* Chemotaxis, Development, and Cell Differentiation.
Rachel L. Rice (Undergraduate)*, and Jeff Hadwiger
Oklahoma State University, Stillwater, Oklahoma

Dictyostelium discoideum is a soil amoeba that undergoes multicellular development when starved. Protein Kinase A (PKA) is known to be involved in this developmental life cycle. Though the full extent of PKA function in *Dictyostelium* is unknown, it is hypothesized that PKA activity promotes many cellular responses, and that this effect should be intensified when the PKA catalytic subunit is overexpressed. PKA overexpression accelerates development and this study examined if this accelerated development occurs in the developmentally delayed *gα5*$^-$ aggregates. Developmental morphogenesis, chemotactic assays, and gene expression experiments were utilized to determine the effects of PKA overexpression. PKA overexpression accelerated development in wild type cells and *gα5*$^-$ mutant. It also decreased chemotaxis to extracellular folate and promoted prestalk gene expression. These results support the hypothesis that PKA overexpression up-regulates many processes in the cell, and may serve a role in promoting prestalk cell differentiation.

Loss of cAMP-specific Phosphodiesterase Rescues Spore Production in *Dictyostelium* G Protein Mutant.
David Schwebs, Hoai-Nghia Nguyen, Jamison Miller, Jeffrey A. Hadwiger.
Oklahoma State University, Stillwater, Oklahoma.

Cyclic-AMP (cAMP) is an important secondary messenger in eukaryotic cells. In *Dictyostelium discoideum*, cAMP also acts as an external signal for cell aggregation and other processes in the developmental life cycle. Previous studies have shown that cells lacking the map kinase ERK2 cannot aggregate and go through development but the disruption of a phosphodiesterase gene, *RegA*, rescues the development of *erk2*$^-$ cells suggesting ERK2 inhibits RegA function. ERK2 is activated in signaling pathways that involve the Gα2 and Gα4 G protein subunits and so RegA regulation is expected in these pathways. It was questioned if a *RegA* gene knockout would rescue phenotypes associated with *gα2*$^-$ or *gα4*$^-$ cells, both of which act upstream of Erk2. The *RegA* gene disruption had only minor effects on the developmental morphology of these mutants but spore production in *gα4*$^-$ aggregates was restored. It was determined that the *RegA* mutation rescues sporulation in a cell autonomous matter. *RegA* gene disruption also provides extracellular signals that lead to an acceleration of prestalk gene expression.

Effector Role of Invasion Plasmid Antigen D (IpaD) of the type III secretion system (T3SS) from *Shigella flexneri*.
Olivia Arizmendi (Doctoral)*[1], Nicholas E. Dickenson[1], Andrew J. Olive[2], William D. Picking[1], Wendy L. Picking[1]. [1]Oklahoma State University, Stillwater, Oklahoma; [2]Harvard Medical School, Boston, Massachusetts.

Shigella flexneri is one of the most common causes of bacillary dysentery. Virulence of this organism depends on its type III secretion system (T3SS), which allows it to invade the colonic epithelium and evade the host immune response. Invasion plasmid antigen D (IpaD) is required for T3SS functionality, as a structural element at the tip of its extracellular needle and by controlling secretion of effectors. We propose IpaD has an undescribed effector role in *S. flexneri*. We transfected a humanized ipaD gene into HEK-293 cells; and IpaD expression was analyzed by western-blot, confocal immunofluorescence (IFM) and co-immunoprecipitation (Co-IP). By IFM, IpaD co-localizes with F-actin and is present throughout the cell periphery; thus, we hypothesize it is interacting with elements of the cytoskeleton. Morphological changes such as formation of cell projections resembling filopodia and lamellipodia were also observed. Co-IP with an anti-IpaD antibody isolated putative binding partners which were identified by mass spectrometry. We are currently analyzing these possible interactions by reverse co-IP, *in vitro* binding and electron microscopy. Our focus is in those with

cytoskeletal roles, as these could explain the phenotype of ectopic expression and be largely significant for *S. flexneri* pathogenesis.

Gelatin- hyaluronic acid scaffolds for intestinal tissue engineering.

Vahid Shabafrooz [a*], Masoud Mozafari [a], Senait Assefa [b],Daryoosh Vashaee [c], Gerwald A. Köhler [b], Lobat Tayebi [a,d].

[a] Helmerich Advanced Technology Research Center, School of Materials Science and Engineering, Oklahoma State University, OK 74106, USA. [b] Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, OK 74107, USA. [c] Helmerich Advanced Technology Research Center, School of Electrical and Computer Engineering, Oklahoma State University, OK 74106, USA. [d] School of Chemical Engineering, Oklahoma State University, Stillwater, OK 74078, USA

Due to the anatomical or functional loss of intestine, in majority of cases, intestinal failure might be occurred, in which the organ does not have its secretory and absorptive functions. Three-dimensional (3-D) porous scaffolds can play a vital role, as they not only serve as temporary templates to guide cell growth and new tissue formation, but also provide sufficient porosity for nutrients and oxygen transport. Scaffolds can be loaded by antibacterial agents too. Ideally, the scaffolding materials should also be a mimic of the natural extracellular matrix (ECM) of the target tissue by integrating suitable mechanical, structural, and biological signals into scaffold. It is known that hyaluronic acid (HA) is an important component of ECM and presents in many biological fluids and tissues. HA has been also demonstrated to have an important role in promoting the penetration of cells into scaffolds due to its high water-holding capacity and intrinsic swelling property in biological media. In this study the gelatin/HA scaffolds have been investigated. The biological compatibility of the prepared scaffolds was evaluated in cultures of Caco-2 cells (human intestinal epithelial cell line). We will discuss the viability of the cells in these scaffolds.

Tissue Engineering Conductive Scaffolds to Employ Bacteriostatic Effect of Electrical Stimuli.

Aref Shahini[1*], Daryoosh Vashaee[1], Lobat Tayebi[2,3]

[1] Helmerich Advanced Technology Research Center, School of Electrical and Computer Engineering, Oklahoma State University, OK 74106, USA. [2] Helmerich Advanced Technology Research Center, School of Materials Science and Engineering, Oklahoma State University, OK 74106, USA. [3] School of Chemical Engineering, Oklahoma State University, Stillwater, OK 74078, USA

Electrical stimulation has been known as a capable tool to facilitate wound healing. It is hypothesized that one of the reasons for this mechanism is the bacteriostatic effect of electrical stimuli. In the other words, such electrical stimulation reduces bacterial growth.

Tissue engineering techniques have recently attracted much attention for wound healing applications. In this regard, if one intends to apply the electrical stimuli to further facilitate the healing procedure, the chemical structure of the tissue engineering scaffolds should be adjusted to locally deliver the electrical stimuli. Non-conductive scaffolds cannot transport the electrical stimuli to the target area. In this study, we focused on manipulating the chemical composition of the tissue engineering scaffolds to increase their conductivity aiming to influence the reproduction of the bacteria under an external electric field. For this purpose we employed a biocompatible conductive polymer namely PEDOT:PSS in the composition of a regular non-conductive scaffold made of gelatin and bioactive glass. The material characteristics of the scaffolds alter significantly by the addition of the conductive polymer. We investigated the morphology, conductivity, swelling rate, biodegradability, and porosity of the new scaffold and concluded that the new scaffolds can still be employed for tissue engineering purposes. For future work, we will study the growth of the various bacteria in these scaffolds at the absence and presence of the electrical stimuli.

Microbial and Molecular Analysis of Morgellons Disease Samples
Lindsey Allen (B.S.)*, Randy S. Wymore, Emily VanDegrift, Jennifer Burkeen, and Rhonda L. Casey. Oklahoma State University-Center for Health Sciences Tulsa, Oklahoma.

Morgellons is a pathology that has been characterized by the production of lesions and subsequent fibers within the epidermis of patients. The causative agent of Morgellons Disease is unknown. Our past and current research has focused on attempts to identify the causative agent of Morgellons Disease. Environmental samples were obtained for analysis from Morgellons affected and non-affected households. Techniques used for analysis in the microbiology laboratory include culturing and isolating of colonies from samples, gram staining, catalase and oxidase testing, with following API testing. A variety of both fungal and bacterial isolates were analyzed. Ensuing DNA extraction, PCR and sequencing were performed on the isolates. Results of this work will describe why certain putative, causative agents of Morgellons can be eliminated and others cannot.

General Microbiology (Convener Sallie Ruskoski)

A Biochemical and Biophysical Characterization of AzoC, the Azoreductase Enzyme of *Clostridium perfringens.*
Jessica Morrison (Doctoral)*, Shuo Dai, Jie Ren, Amanda Taylor, Mitchell Wilkerson, Cristee Wright, Aihua Xie and Gilbert John. Oklahoma State University, Stillwater, OK.

Azo dyes are used widely across industries as colorants. Many microorganisms are able to reduce azo dyes by use of an azoreductase enzyme and through the reduction of the azo bonds of the dyes that carcinogenic metabolites are produced. The field of research on azoreductases is growing, but there is very little information available on azoreductases of strictly anaerobic bacteria. The azoreductase gene was identified in *Clostridium perfringens* (AzoC), a strict anaerobe that is found in human intestinal tracts. AzoC was biochemically characterized via UV-VIS spectroscopy and was found to have high activity, especially with Direct Blue 15. AzoC was found to work best at pH 9.0, 25C, and with NADH and FAD as cofactors. AzoC was biophysically characterized using Mass Spectroscopy, FTIR, Circular Dichroism, and SDS PAGE. FAD was identified as the non-covalently bound cofactor of AzoC in a 1:1 ratio. By SDS-PAGE, AzoC was determined to be a trimer connected by disulfide bonds. The trimeric form does not seen to add to structural stability, as determined by thermal melt studies. Computational analysis showed the secondary structure of AzoC is consistent with the structural characteristics of other azoreductases, suggesting that gut enzymes of similar function will have related structures.

Role of RND Transport Systems in Calcium-Induced Antibiotic Resistance and Virulence in *Pseudomonas aeruginosa*.
Sharmily S Khanam* (Doctoral), Dirk L. Lenaburg, Ryan C. Kubat, and Marianna A. Patrauchan. Oklahoma State University, Stillwater, OK.

Pseudomonas aeruginosa is a facultative pathogen infecting lungs of cystic fibrosis patients, causing infective endocarditis and severe implant infections. It is highly adaptable and demonstrates resistance to practically all antimicrobials available for clinical treatments. Calcium (Ca^{2+}) is a well-established signaling molecule that regulates essential processes in eukaryotes including innate immune responses. Earlier we showed that Ca^{2+} triggers biofilm formation and virulence in *P. aeruginosa.* We determined that Ca^{2+} increases minimal inhibitory concentrations of tobramycin and polymixin B at least tenfold in *P. aeruginosa* PAO1. Proteomic analyses of PAO1 membrane and extracellular proteins using LC-MS/MS-based spectrum counting identified

four multidrug efflux pumps MexAB-OprM, MexGHI-OpmD, TriABC-OpmD and MuxABC-OpmB that were significantly affected by Ca^{2+}. To study the role of RND systems in Ca^{2+}- induced antibiotic resistance and virulence, we used transposon mutants with disrupted RND systems (11) identified in the PAO1 genome. The results revealed that five RND systems *mexB*, *mexC*, *mexV*, *mexE*, and *czcB* play role in Ca^{2+} induced tobramycin resistance, whereas all eleven RND systems play role in Ca^{2+}-induced virulence. This suggests that RND transport systems contribute to Ca^{2+}- induced adaptation mechanisms in *P. aeruginosa*, and may provide additional protection against antimicrobials and host defenses in response to elevated Ca^{2+}.

Three Functional β-Carbonic Anhydrases in *P. aeruginosa* PAO1. Role in Survival in Ambient Air and Calcification.

Shalaka R. Lotlikar (Doctoral)*, Shane B. Hnatusko, Wendy L. Picking, and Marianna A. Patrauchan. Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

Calcium (Ca^{2+}) plays a key regulatory role in eukaryotic processes. Abnormalities in Ca^{2+} homeostasis may lead to soft tissue calcification commonly associated with human diseases. However the origin and mechanisms of such calcification remain unknown. Our hypothesis is that *P. aeruginosa* PAO1 carbonic anhydrases (CAs) play role in $CaCO_3$ deposition. Quantitative analysis of Ca^{2+} showed that PAO1 precipitates 0.12 and 0.35 mM Ca^{2+} when grown at 1 and 5 mM Ca^{2+}, respectively. Transmission electron microscopy followed by X-Ray elemental analysis confirmed the formation of 0.08-0.1 µm crystals containing Ca^{2+} during PAO1 growth at 10mM Ca^{2+}. Bioinformatic analysis identified three genes PA0102, PA2053, PA4676 encoding cytosolic β-CAs in PAO1 genome. CAs were purified and confirmed to have specific CA activity. Immunoblot analysis showed that all CAs are expressed in PAO1 cells when grown in ambient air and 5% CO_2, PA0102 appeared more abundant under both conditions. Growth studies of transposon mutants showed that the disruption of *PA0102* impaired PAO1 growth in ambient air and caused a minor defect at high CO_2. Thus, PA0102 contributes to the adaptation of *P. aeruginosa* to low CO_2 conditions and will be further studied for its role in calcification and virulence.

Calcium Homeostasis in *Pseudomonas aeruginosa* Requires Mutliple Transporters

Manita Guragain (Doctoral), Dirk L. Lenaburg, Frank S. Moore, Ian R. Reutlinger, Marianna A. Patrauchan*
Oklahoma State University, Stillwater, OK

Calcium (Ca^{2+}) homeostasis is tightly regulated in eukaryotes, and plays an important role in numerous essential cellular processes. Although certain bacteria are also capable of maintaining Ca^{2+} homeostasis, little is known about the underlying mechanisms. *Pseudomonas aeruginosa* PA01, a facultative human pathogen causing severe acute and chronic infections, responds to elevated Ca^{2+} by enhancing biofilm formation and production of virulence factors. By using Ca^{2+}-binding photoprotein aequorin, we have measured the concentration of free intracellular Ca^{2+} ($[Ca^{2+}]_{in}$) in PAO1 to be 0.14±0.05 µM. Upon adding 1 mM Ca^{2+}, $[Ca^{2+}]_{in}$ increased 13 fold and declined. Growth at elevated Ca^{2+} increased the efflux rate of $[Ca^{2+}]_{in}$. Treatment with inhibitors suggests the role of ion exchangers, ATP synthases, and P-type ATPases in Ca^{2+} homeostasis. To identify Ca^{2+} transporters in PAO1, LC-MS/MS peptide counting was used. Seven Ca^{2+}-induced and eleven bioinformatically predicted transporters were targeted for monitoring $[Ca^{2+}]_{in}$ by using transposon insertion mutants (UW Genome Center). Thirteen mutants showed changes in $[Ca^{2+}]_{in}$ response, and disruption of P-type ATPases PA2435, PA3920, and ion exchanger PA2092 impaired Ca^{2+} homeostasis. All the mutants showed high tolerance to external Ca^{2+}. This apparent redundancy indicates the importance of Ca^{2+} homeostasis in *P. aeruginosa* physiology and requires further studies.

Prevalence of Plasmids in *Campylobacter* spp. Isolated From Oklahoma Retail Meats.

Daya Marasini (Doctoral)*, and Mohamed K. Fakhr. Department of Biological Science, The University of Tulsa, Tulsa, Oklahoma.

Campylobacter spp. is one of the most frequently prevalent bacterial pathogens in food and is emerging as a leading cause of diarrhea in humans. Very few studies have been published discussing the role of plasmids in *Campylobacter*. The literature is lacking in particular studies related to *Campylobacter* mega plasmids. The objective of this study was to determine the prevalence of plasmids particularly the mega ones in *Campylobacter* spp. isolated from Oklahoma retail meats. Plasmids were isolated from 189 *Campylobacter jejuni* and *Campylobacter coli* strains by alkaline lyses methods. Pulsed Field Gel Electrophoresis was used to isolate the larger sized mega plasmids from the *Campylobacter* spp. strains. Out of 189 strains, 99 (52.38%) were found to harbor plasmids via the alkaline lyses method. Most of the plasmids were smaller than 65 Kb in size where only two were about 90 Kb. *Campylobacter coli* were found to have more prevalence of plasmids than *Campylobacter jejuni.* Turkey isolates were found to have higher plasmid prevalence than isolates from chicken, pork and gizzard samples. The PFGE method was able to isolate plasmids of larger size up to 180 Kb. Most of the larger plasmids were found to be present in *Campylobacter coli*.

Green Synthesis of Gelatin Based Antibacterial Macroporous Hybrid Scaffolds of Gelatin/Nanosilver/Bioactive Glass for Bone Tissue Engineering.

Mostafa YazdiMamaghani[1*], Senait Assefa [2], Daryoosh Vashaee [3], Gerwald A. Köhler [2], Lobat Tayebi [1,4]
[1]Helmerich Advanced Technology Research Center, School of Material Science and Engineering, Oklahoma State University, Tulsa, OK. [2]Department of Biochemistry and Microbiology, Oklahoma State University Center for Health Sciences, Tulsa, OK. [3]Helmerich Advanced Technology Research Center, School of Electrical and Computer Engineering, Oklahoma State University, Tulsa, OK. [4]School of Chemical Engineering, Oklahoma State University, Stillwater, OK.

It has been estimated that about sixty percent of infections in hospitals are related to biofilms. Due the excessive use of antibiotics, multi-drug resistant bacterial strains have emerged, which show remarkable resistance to conventional antibiotics and biocides. Among the various antibacterial materials, silver nanoparticles have attracted intensive research interest. They possess an intrinsic antibacterial activity against a broad spectrum of pathogenic microorganisms with preventability of antimicrobial resistance. This work presents the environmentally-friendly synthesis of silver nanoparticles through heat-treatment within the gelatin solution with the purpose of making a hybrid gelatin/bioactive glass/nanosilver macroporous scaffold for bone tissue engineering applications. The effects of the incorporation of silver nanoparticles in different concentrations in the scaffolds on antimicrobial properties were investigated. As indicators for antimicrobial effects, two species of prokaryotic organisms (*Escherichia coli*, *Staphylococcus aureus*), and one eukaryotic species, the opportunistic pathogenic fungi *Candida albicans* were studied. The prepared nanosilver-containing scaffolds presented high antibacterial activity towards both gram-positive and gram-negative bacteria.

Environmental Microbiology (Convener Dr. Sue Katz)

Evaluation of the Phylogenetic Diversity and Community Structure of Members of Candidate Phylum OP8 Using *in silico* Data Mining Approaches

Ibrahim F. Farag (Doctoral Student)*, James P. Davis, Noha H. Youssef, and Mostafa S. Elshahed
Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74074, USA.

Utilization of culture-independent 16S rRNA gene-based diversity surveys has lead to the identification of numerous yet-uncultured bacterial phyla in nature. With the exception of their origin and 16S rRNA gene

sequence, very little is currently known regarding the metabolic capabilities, physiological characteristics, and ecological roles of members of such phyla. We are conducting a thorough evaluation of the phylogenetic diversity, distribution, and community structure of members of candidate phylum OP8 (CP-OP8), initially identified in a thermal pool in Yellowstone National Park. We provide an updated taxonomic outline of CP-OP8 based on available near full-length 16S rRNA gene sequences in public databases. We also investigated the prevalence of CP-OP8 in publicly available next generation (pyrosequencing and Illumina) 16S datasets. Analysis of 1.8 billion partial 16S rRNA gene sequence identified 47351 CP-OP8 sequences in 913 (33.4%) of datasets examined. Abundance of OP8 members ranged between 0.00015 and 10.2%, and was highest in marine, followed by fresh water and soil. Statistical analysis identified distinct CD-OP8 community structure patterns, with members of OPB95 order mostly affiliated with petroleum ecosystems and OP_GUA class mostly affiliated with hydrothermal vents. The results represent the first systematic evaluation of the diversity, distribution, and community structure of candidate division OP8 on a global scale.

Genomic Analysis of Hydrocarbon Degradation in *Arhodomonas* sp. strain Seminole.

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Several studies have reported the ability of halophilic and halotolerant organisms to degrade hydrocarbons for their potential use in bioremediation of polluted hypersaline environments. We have isolated novel halophiles, *Arhodomonas* sp. strain Seminole and strain Rozel that degrade benzene and toluene as the sole carbon source at high salinity (1 to 4 M NaCl). A high quality draft genome of strain Seminole was obtained to gain insights into the physiology of these halophiles. It was analyzed for genes involved in aromatic hydrocarbon degradation. To corroborate the genomic data, the strains were grown on a range of aromatic compounds. Genomic analysis revealed the presence of ring-hydroxylating monooxygenases and dioxygenases that catalyze the initial rate-limiting steps in the degradation pathways. In addition, a variety of ring-cleaving genes such as catechol 1, 2-dioxygenase, catechol 2, 3-dioxygenase, protocatechate 3, 4-dioxygenase, and homogentisate 1, 2-dioxygenase were also identified. Microcosm studies revealed that both strains are capable of using catechol, protocatechuic acid, gentisic acid, 4-hydroxybenzoic acid or benzoate as growth substrates. In this study, we have identified the catabolic potential of the strains and the pathways for metabolizing hydrocarbons. Such studies are important for understanding degradation mechanisms and developing effective bioremediation strategies for contaminated saline environments.

Genome Sequence of the Anaerobic Gut Fungi *Orpinomyces* sp. strain C1A

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Anaerobic gut fungi (AF) represent a distinct basal fungal phylum (Neocallimastigomycota), and reside in the rumen and gut of herbivores. AF play an important role in plant biomass degradation in ruminant herbivores, represent the only strictly anaerobic fungal group, and are efficient colonizers of ingested plant materials in cow rumens. Here, we report on the sequencing and analysis of he genome of the AF *Orpinomyces* sp. strain C1A. The genome was sequenced using a combination of Illumina and PacBio SMRT technologies. The large fungal genome (100.95 Mb, 16,347 genes) displayed extremely low G+C content (17.0%), large non-coding intergenic regions (73.1%) and proliferation of microsatellite repeats (4.9%). Analysis of the lignocellulolytic machinery revealed an extremely rich repertoire of CAZy enzymes, with evidence of horizontal gene acquisition from multiple rumen prokaryotic lineages. The genome contained all genes required for the degradation of cellulose, as well as xylans mannans, and mixed β-glucans. The genome appears to be highly adapted to the degradation of xylans, the prevalent hemicelluloses in grasses (order Poales). We argue

that the unique features in the genome, when compared to other members of the Mycota, is a reflection of their distinct phylogenetic position and unique evolutionary trajectory.

Cell Envelope Permeability Properties for Nonpolar Compounds in Bacteria Isolated from Waste Water Effluents

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The hydrophobic biocide triclosan is a stable, broad-spectrum compound commonly used in health and personal care products to inhibit bacterial growth. Leaching from these products into effluent waste water could enrich for resistant soil bacteria, thereby leading to cross-resistance to other antimicrobial agents. The purpose of this study was to determine the relative susceptibility of environmental bacteria selected in the absence and presence of triclosan to both triclosan and the mechanically-disparate hydrophobic antibiotic novobiocin. 16S rRNA sequencing revealed all organisms isolated in the presence of triclosan were most closely related to the genus *Pseudomonas* and exhibited resistance to both hydrophobic molecules in a manner similar to that of the refractory soil organism *Pseudomonas aeruginosa*. Organisms related to *Flavobacterium* spp., *Rheinheimera* spp., and *Acidovorax* spp. were isolated in the absence of triclosan and exhibited intrinsic susceptibility to both hydrophobic antibiotics. Of two *Pseudomonas* spp. isolated in the absence of triclosan, one was resistant to triclosan and novobiocin, while the other exhibited susceptibility to both. These data suggest a component(s) of treated waste water effluent (most likely triclosan) selects for bacteria related to *Pseudomonas* spp. that are resistant to nonpolar compounds by virtue of outer membrane exclusion.

Medical Microbiology/Immunology (Convener Dr. Gerwald Köhler)

Biophysical characterization for IpaD-IpaB fusion complex from *Shigella flexneri* as a candidate subunit vaccine.

Xiaotong Chen (doctoral)*, Nick Dickenson, Francisco X Martinez-Becerra, Shyamal P Choudhari, Jamie C Greenwood II, William D Picking and Wendy L Picking. Department of Microbiology, Oklahoma State University, Stillwater, OK

Shigella are a causative agent of gastrointestinal illness and are responsible for high morbidity among the elderly and children. *Shigella* infects the host using a type III secretion system (T3SS), Two component of the exposed needle tip complex of T3SS, IpaD and IpaB, have been shown to be potential broadly protective antigens in the mouse lethal pneumonia model. The DB fusion needs to be co-expressed with IpaB's cognate chaperone, IpgC which is then removed from the fusion complex with the mild detergent OPOE and the pure DB fusion is used for biophysical characterization. We subsequently constructed an empirical phase diagram (EPD) that is used to determine the physical state of the protein as a function of both temperature and pH. From the EPD data we found that the DB fusion is most stable at around pH 7 below 35° C. Another mild detergent LDAO was also used in the purification instead of detergent OPOE and we found that LDAO brings more stability to the fusion protein compared to the OPOE. Based on the EPDs, we will identify the desired conditions for excipient screening studies.

Biophysical characterization and stabilization of CagL, an antigenic protein from *Helicobater pylori* as a candidate subunit vaccine

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Protein-biopharmaceutical agents are prone to degradation over time, so it is important to determine optimal conditions that allow retention of protein integrity and biological activity. The protein CagL, present in strains of the bacterium *Helicobacter pylori* possessing type IV secretion systems, is encoded by the Cag Pathogenicity Island and is an attractive candidate for vaccine development against *H. pylori*. In this study, CagL was subjected to biophysical characterization using different spectroscopic techniques such as near- and far- UV circular dichroism, extrinsic fluorescence and light scattering to assess physical stability under different pH and temperature conditions. The stability at each pH condition was determined in terms of transition temperature (Tm) value. The data generated from characterization studies could be used to generate an empirical phase diagram (EPD). This EPD depicted the different physical states for this protein under relevant stress conditions and allowed us to select stress conditions that could be used for the screening of excipients (stabilizers) that will prevent protein aggregation. Potential excipients were screened and tested to determine their enhanced stabilizing effects in terms of increased transition temperature. These analyses will help in formulation of an effective and stable vaccine against *H. pylori.*

Evaluating the Antibody Response Following Experimental Infection with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV).

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PRRS is the most economically important swine viral disease. The neutralizing antibody (nAb) response following PRRSV infection is weak and delayed, and primarily directed against the homologous virus. The purpose of this study is to evaluate the nAb response in a large population of experimentally infected pigs. Based on studies of HIV, we predict that a population of pigs exists that produce nAb to a broad range of isolates (bnAb). Approximately 1,200 samples,collected 42 days after PRRSV challenge, were assayed for neutralizing activity against four genetically diverse PRRSV isolates. Samples were placed into the following groups (Grp): Grp1, no neutralizing activity; Grp2, neutralizing activity against only the homologous virus; Grp3, neutralizing activity against the homologous virus and one or two additional isolates; or Grp4, neutralizing activity against all four isolates. Virus neutralizing activity has a genetically heritable component. There is also an inverse relationship between virus load and homologous nAb titer, indicating a role for nAb in the control of virus replication. The identification of pigs showing a bnAb response suggests the existence of conserved neutralizing epitopes. The genetic basis for a bnAb response creates the opportunity to develop pig lines that are tailored to respond optimally to PRRS vaccines.

Protective Efficacy of SPI-1 and SPI-2 Proteins in a Mouse *Salmonella* Model

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Salmonella enterica, with over 2500 serovars, causes nearly 1.3 billion cases of disease and mortality annually in both humans and animals. Serovar Typhi causes enteric fever while non-typhoidal serovars such as Typhimurium cause gastroenteritis. In spite of high numbers of infection, a universal vaccine against many serovars is still absent. Two of the *Salmonella* pathogenicity islands encode type three secretion systems, SPI-1

and SPI-2, which increase virulence and pathogenicity. Previous studies utilized *Shigella* T3SA proteins IpaB and IpaD as protective antigens in mouse lethal pulmonary models, leading us to investigate the protective efficacy of homologous proteins in *Salmonella*. Mice were immunized intranasally with either the SPI-1 proteins SipB and SipD or the SPI-2 protein SseB, with either MPL or dmLT as the adjuvant. Immunogenicity was tested through antibody generation, enumerating ASCs and cytokine secretion. Mice were then challenged with lethal doses of *S.* Typhimurium administered orogastric or *S.* Typhi administered intraperitoneally. We found that SPI-1 proteins SipB and SipD were effective against a challenge with *S.* Typhi while the SPI-2 protein SseB was effective against *S.* Typhimurium. Our results indicate the differential roles these proteins serve during infection with each serovar, providing information for further development of broad-range *Salmonella* vaccines.

Characterizing the Protein-Lipid Interaction of Invasion Plasmid Antigen B (IpaB) from *Shigella flexneri*

Philip R. Adam (Doctoral)*, Wendy L. Picking, and William D. Picking. Oklahoma State University, Stillwater, OK

Shigella flexneri is a Gram-negative pathogen that uses its type III secretion system (T3SS) to invade host colonic epithelial cells. The T3SS is comprised of a basal body anchored within the inner and outer membranes, a surface exposed needle, and a complex of proteins on the tip of the needle. This tip complex is responsible for environmental sensing and secretion control. Invasion plasmid antigen B (IpaB) is part of the T3SS tip complex. We have found that oligomeric IpaB interacts with and lyses liposomes *in vitro* while monomeric IpaB still interacts with but cannot disrupt liposomes. We asked the question: What regions of IpaB are involved in the protein:lipid interaction interface? Single Cys substitutions were made throughout the IpaB sequence and these sites were labeled with fluorescein. The modified proteins were then used in fluorescence quenching experiments to determine the differential solvent accessibility of each residue for monomeric and oligomeric IpaB in the presence and absence of liposomes. Quenching experiments suggested the hydrophobic portion of IpaB was protected from solvent by liposomes, especially as an oligomer. These data are helping us refine our developing model of how IpaB oligomers contribute to the generation of the *Shigella* T3SS translocon pore.

Identification of probiotic genes of lactobacilli isolated from prairie voles using next generation sequencing.

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Probiotics show potential for treatment of both neurological disorders and heavy metal poisoning. The highly social prairie vole is an excellent model in which to study the impact of environmental factors, such as heavy metal ingestion, on behavior. Previous studies showed that exposure to mercury chloride causes antisocial behavior in prairie voles. The use of enrichment media for lactobacilli and a range of *in vitro* tests for probiotic effects led to the isolation of thirty *Lactobacillus* strains with probiotic potential from the prairie vole intestine. All strains were closely related to *Lactobacillus johnsonii*, according to 16S rRNA gene sequences. *In vitro* assays assessed probiotic characteristics such as the ability to survive passage through the stomach and colonize the gastrointestinal tract. The strains were tested for acid tolerance, resistance to bile and bile salts, as well as antimicrobial potential against fungi and bacteria. Adherence to intestinal epithelial cells and resistance to mercury chloride were also examined. Two strains which showed high probiotic potential were chosen and their genomes sequenced using an Ion PGM sequencer. Using comparative genomics analysis the sequences will be further evaluated for genes responsible for probiotic qualities

Desulfovibrio spp. and Mercury Methylation in the Gastrointestinal Tract of Prairie Voles.

David J. Supeck*[1], Senait Assefa[1], Yue Chen[2], J. Thomas Curtis[2], and Gerwald A. Köhler[1]. [1]Department of Biochemistry & Microbiology, [2]Department of Pharmacology & Physiology, Oklahoma State University Center for Health Sciences, Tulsa, Oklahoma.

Mercury is a heavy metal known to alter the social behavior in the highly social prairie voles. We are trying to correlate the effects of mercury on vole social behavior with changes in the composition of the gut microbiota (gut-brain axis). Adult prairie voles received $HgCl_2$ in drinking water *ad libitum* for ten weeks while control voles received unadulterated water. The intestinal microbiotas were characterized using pyrosequencing and quantitative real-Time PCR. Male prairie voles exposed to $HgCl_2$ displayed reduced interaction with unfamiliar conspecifics while female prairie voles appeared to be unaffected. Compositional analyses of the microbiotas of male/female and treated/untreated animals revealed differences in some genera/species including *Desulfovibrio* species which were present in higher numbers in mercury-treated voles. Because of the integration of the intestinal microbiota in the gut-brain axis, the observed effects of toxic metal exposure on vole behavior might also be based on changes in the microbiota. We have identified sex-specific differences which could play a role in the increased susceptibility of male voles to the behavior-altering effects of mercury-ingestion. *Desulfovibrio spp.* might increase the bioavailability of mercury through methylation. Future studies will be directed towards elucidation of the molecular mechanisms of mercury toxicity and the role of the gut microbiota in social behavior.

Effect of Monosaccharide Supplementation on Biofilm Formation in Variant Strains of _Burkholderia multivorans_

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Burkholderia multivorans is a gram-negative bacillus that causes opportunistic pulmonary infections in patients with underlying diseases. The purpose of the present study was to examine the effect of disparate sugars on the propensity to form biofilms in phenotypically variant *B. multivorans* strains. Yeast Extract broth (YEB) containing a single sugar supplement and conventional 96-well microtiter plate assays were employed to assess adherence, biofilm maturation, and total sugar concentrations. Mannose supplementation facilitated biofilm growth at 24 and 48 hours by a highly mucoid strain and two non-mucoid derivatives as compared to control growth with YEB alone, as well as with either ribitol or sorbose supplementation of YEB. Other strains produced less biomass at 48 hours than at 24 hours, thereby suggesting that the maturing biofilm biomasses were less stable. Additionally, all strains underwent increases in total sugar concentration in the biomass indicating possible involvement of extracellular polysaccharides (EPS) in biofilm formation. Microscopic observations of negatively-stained biofilms revealed growth in the presence of mannose resulted in denser, more populated biomasses having more non-cellular EPS present around microcolonies than did mannose-deficient control biofilms. These data support the conclusion that mannose supplementation enhances biofilm formation in the mucoid *B. multivorans* strain and its derived variants, thereby creating a denser, more stable biomass with maturation.

Undergraduate and High School Presentations (Convener Dr. Cindy Cisar)

Characterization of the Putative DnaK-suppressor Protein in the Lyme Disease Spirochete *Borrelia burgdorferi*.

Tia Hadley (undergraduate) and Travis J. Bourret. Department of Biology, University of Nebraska at Kearney, Kearney, NE.

Borrelia burgdorferi is the causative agent for Lyme disease. *Borrelia* encounters many environmental challenges including shifts in temperature, pH, osmolarity, nutrient availability, as well as, oxidative and nitrosative stresses throughout its natural infectious cycle in *Ixodes spp.* ticks, birds, the white-footed mouse, white-tailed deer, dogs, cats, humans, and other small mammals. Many bacteria respond to environmental challenges by conserving energy using the stringent response mediated by ppGpp(p) and the DnaK-suppressor protein (DksA). DksA coordinates the interaction of ppGpp(p) with RNA polymerase resulting in the down-regulation of transcriptional and translational machinery gene expression. *B. burgdorferi* strain B31 open-reading frame *bb0168* encodes a putative *dksA* homologue. We set out to characterize *bb0168* using a heterologous system in *Escherichia coli.* This was achieved by cloning *bb0168* into the arabinose-inducible plasmid pBAD/HisA to form the vector pBdksA. This vector was introduced to a *ΔdksA* mutant in *E. coli* strain BW25113, which is hypersensitive to killing by 0.5 mM hydrogen peroxide (H_2O_2) and displays a growth defect in minimal M9 salts + 0.2% casmino acids (CAA) compared to wild-type controls. We have shown that *in trans* expression of *bb0168* from the pBdksA plasmid functionally complemented the *ΔdksA E. coli* strain restoring wild-type levels of resistance to H_2O_2 and growth in M9 + CAA growth medium. Collectively, these data indicate that *bb0168* encodes a functional *dksA* allele that may be crucial for the stringent response in *B. burgdorferi*.

Comparative In Vitro Antimicrobial Efficacies of Polyphenol Extracts on Non-pathogenic Bacteria and MRSA and MSSA.

J. Gage Holleman (High School Student)*. Cascia Hall Preparatory School, Tulsa, Oklahoma.

Polyphenol extracts show a novel solution to the growing multi-drug resistance problem in bacteria. This research provides evidence for the potential of green tea, grape seed extract, and propolis to inhibit bacterial growth, increase antibiotic sensitivity, and inhibit the formation of biofilms. Concentrations of 10% ethanol extracts of polyphenols were evaluated for efficacy in dilutions to 1×10^{-5}. Cultures of *E. coli, B. subtilis*, MRSA, and MSSA were evaluated. 24 hour cultures, OD 0.1, were plated on Mueller-Hinton Agar and each polyphenol was evaluated for MIC using a standard macrodilution assay. Bacteria were evaluated for antibiotic sensitivity to chloramphenicol, penicillin, vancomycin, erythromycin, tetracycline, and neomycin. Sensitivity was evaluated using polyphenol enhanced MH plates for synergistic effects. Polyphenol extracts were diluted in a 96 well plate, inoculated with bacteria (OD 0.1), incubated (37°C for 24 hours) to determine the effects of polyphenols on biofilms using a standard crystal violet assay and a plate reader (600nm). Significance (Student T-tests), p value less than 0.05, were determined for 21 of the test comparisons for antibiotic sensitivity. All results indicate the presence of polyphenols in the agar increased the efficacy of the antibiotics tested and inhibited the formation of biofilms.

A Phylogenetic Analysis of Bacteria in Western Nebraska Alkaline-Saline Lakes Using an Afex Pretreated Cellulose Enrichment

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Department of Biology – University of Nebraska at Kearney

In the Sandhills of western Nebraska, alkaline-saline lakes are common. These lakes are important because the microorganisms found in these environments turnover carbon very quickly. Nylon bags containing Afex pretreated cellulose were placed in three different alkaline-saline lakes in Western Nebraska: Border Lake (highly alkaline-saline), Kokjohn Pond (highly alkaline-saline), and Tree Claim Lake (moderately alkaline-saline). After three months in the lakes, resultant bacterial communities were sent for next generation DNA sequencing. Phylogenetic analysis was conducted using the Ribosomal Database Project Pyrosequencing Pipeline. Proteobacteria was the predominant phylum present at all three locations. Border Lake and Kokjohn Pond (highly alkaline-saline) contained similar phylogenetic compositions, while Tree Claim Lake (moderately alkaline-saline) demonstrated greater phylogenetic diversity. This study aims to determine the phyla in the environment that are responsible for the rapid carbon turn over in these alkaline-saline lakes.

Identification of Siderophore Producing Bacteria from Alkaline-Saline Lakes

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Western Nebraska contains thousands of alkaline-saline lakes that are shallow and iron deficient. These lakes range in pH from 7 to 11. This unusual environment is home to a diverse bacterial population. Despite these extreme conditions, alkaline lakes are regarded as being amongst the world's most productive aquatic environments, even though most of the iron is oxidized. The purpose of this study is to investigate and identify bacteria that produce siderophores, iron scavenging compounds. Siderophore producing bacteria were isolated from the lakes, and then characterized for salt and pH tolerance, gram stain, and oxidase reaction. Quick tests: BBL Oxi/Ferm Tubes, API 20 NE tests, and API ZYM tests, were used to identify biochemical reactions. Six isolates were characterized. All six were gram negative, oxidase positive rods. Using the API 20 NE test, isolates from the alkaline lakes were closely related to *Pseudomonas sp., Ochrobacterium sp.,* and *Burkholderia cepacia.* This agreed with 16S rDNA sequencing results. The next step is to look at DNA G-C content to further identify the bacteria. Once these tests are complete the bacteria can be classified to species, and this information will help us to identify potential siderophore families.

Characterization of Azoreductase Activity of Whole Cell *Enterococcus faecium* under Aerobic and Anaerobic Conditions.

Keely Redhage (Undergraduate)*, Lindsey Berger, Jantzen Matli, Jessica Morrison, Gilbert John. Oklahoma State University, Stillwater, Oklahoma.

Comensal bacteria within the gut, such as *Enterococcus faecium*, can lead to carcinogenic by products when cleaving the double bond of azo dyes. These dyes are present in a variety of products used every day, such as soft drinks, staining in lab, and as pH indicators. This study involves understanding the interactions of *E. faecium* on the selected azo dyes: Direct Blue 15, Tartrazine, Janus Green, Methyl Orange, and Methyl Red which were selected based on size and sulfonation. The objective of this study was to answer three questions: first, what effect does *E. faecium* have on the reduction of the dyes? second, does the size and sulfonation of the dye have an effect on its rate of reduction? and third, what effect does oxygen have in dye reduction? In order to answer these questions the bacterium was inoculated in both aerobic and anaerobic environments. The dyes were then introduced to *E. faecium* and dye absorbance was recorded every hour for four hours, using a UV-

VIS spectrophotometer. The results suggest size and sulfonation has a limited effect on dye reduction. This study provides a better understanding of the bacterium *E. faecium* and its interactions with azo dyes.

Examination of Type III Toxin-Chaperone Interaction by Copurification

Nicholas Kinkead (Undergraduate)*, Amy Lingel, Michael Paz, Dr. Donald Rowen. University of Nebraska Omaha, Omaha, Nebraska

Type Three Secretion systems (TTSS) are an important virulence factor for many pathogenic bacteria including *Pseudomonas aeruginosa*. The secretion of some toxins requires a chaperone protein to bind to the toxin, but the exact role of the chaperone is not completely understood. Previous results have suggested that the *P. aeruginosa* Type III toxin ExoU may be unusual in that residues near both the amino and carboxy-terminus are required for chaperone interaction. The C-terminus of ExoU contains a membrane localization domain (MLD), and chaperones have been hypothesized to act to mask MLDs. To confirm the importance of the C-terminal MLD of ExoU on SpcU interaction, I am seeking to test the ability of ExoU C-terminus truncation mutants to bind to a His-tagged SpcU in a copurification experiment. For that experiment, I have constructed plasmids that will express His tagged SpcU and either wild type or truncated forms of ExoU in *E. coli* cells. I have conducted trial experiment to optimize the conditions for expression and fractionation, and am about to perform the copurification experiment. Lack of purification of truncated forms of ExoU along with SpcU would support the hypothesis that SpcU binds to the C-terminus and N-terminus of ExoU.

Genetic Variation in the *cmeR* Gene Among *Campylobacter coli* Strains Isolated From Turkey, Swine, and Cattle.

Mohamed K. Fakhr[1, 2], Anita Chaphekar[1] (Undergraduate)*, and Catherine M. Logue[2, 3]. [1]Department of Biological Science, The University of Tulsa, Tulsa, Oklahoma. [2]Department of Veterinary and Microbiological Sciences, North Dakota State University, Fargo, North Dakota. [3]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, Iowa.

Campylobacter is one of the leading causes of foodborne diseases in the US. *Campylobacter jejuni* uses an efflux pump system to confer antimicrobial resistance to few antibiotics. The efflux pump, known as cmeABC, is made up of three components. cmeA is the periplasmic fusion protein, cmeB is the inner membrane efflux transporter, and cmeC is the outer membrane protein. The cmeR gene is located upstream of the efflux pump operon and acts as a repressor for the cmeABC operon. While DNA sequences of the efflux pump genes are available for *Campylobacter jejuni*, the GenBank is lacking sequences for *Campylobacter coli* strains. The objective of this study was to sequence the cmeR gene to identify any genetic diversity among more than one hundred *Campylobacter coli* strains isolated from turkey, swine, and cattle. Gene amplification and DNA sequencing of the cmeR gene revealed limited sequence variation among the tested *Campylobacter coli* isolates but distinct from the published *C. jejuni* sequences. Our results also showed a C to T transition mutation in the cmeR binding site between cmeR and cmeA genes that is known to activate the transcription of the efflux pump operon in three of the screened isolates.

Fungal Diversity of a Cottonwood Root System

Jeff A. Shaw (undergraduate)*[1], Mary J. Harner[1,2] and Dawn M. Simon[1]. [1]University of Nebraska-Kearney, NE; [2]Crane Trust, Wood River, NE.

Mycorrhizal fungi are symbiotic partners of plants that facilitate nutrient uptake. Historically, identification has been based primarily on morphology, but better estimates of diversity can be obtained using molecular techniques. In our research, DNA was extracted from four spatially distinct locations within the root system of a

single cottonwood tree (*Populus* spp.) that vary considerably in soil characteristics. Using PCR, a region of the fungal ribosomal RNA was amplified, cloned, and sequenced. We sequenced 135 clones from these four sites and used phylogenetic analyses to determine diversity. The sequences fall into two broad categories: ectomycorrhizal fungi and general soil fungi. Species richness of the mycorrhizal fungi in this system was estimated using the Chao1 index. Rarefraction curves based on this suggest that we have reached saturation with respect to species diversity in this system and that our samples adequately reflect the fungal assemblage at these sites. The sequences of ectomycorrhizial fungi were grouped into more than 10 clusters, with each cluster approximating a distinct species. However, these clusters are closely related, with a nearest BLAST match in GenBank to the same environmental sample. This suggests that there is diversity in mycorrhizae that is not yet represented in GenBank sequences.

Growth and Glutathione Levels of *Enterococcus faecium* in Response to Quinone Exposure.

Bryan R. Fritz (Undergraduate)*, Jessica Morrison, and Gilbert H. John. Oklahoma State University, Stillwater, Oklahoma.

For their size, bacteria perform a vast number of processes. Some bacteria that possess azoreductase activity also have been shown to possess quinone reductase activity. Quinones are compounds with one or more conjugated rings and two carbonyl groups. Derivatives of these compounds serve as important biomolecules, such as ubiquinone in the respiratory chain. Quinones can become a problem due to their potential to cause a buildup of reactive oxygen species. They can also react with cellular thiols, which are needed for cysteine and cofactors, and severely limit their availability to the cell. Therefore, an enzyme with quinone reductase activity would be able to detoxify quinones if they became a threat. To determine if the bacterium *E. faecuim* possesses quinone reductase activity, a culture was grown overnight then exposed to three quinone compounds (naphthoquinone, menadione and lawsone) and one metabolite (1,4 – dihydroxynaphalene, metabolite of naphthoquinone). Growth was monitored by following the absorbance at 600nm over a five hour time frame and results graphed. Glutathione levels are monitored as a way of seeing the cell's response to oxidative stress. This research may help understand the response of *E. faecium* in response to oxidative stress.

Differential Response of Animal and Environmental Isolates of *Escherichia coli* to Bovine Bile (Ox gal), Deoxycholate, pH, incubation temperature and NaCl.

Jessica M. Robertson (undergrad)* and Al T. Mikell, Jr. Oklahoma Christian University, Edmond Oklahoma

Public health standard methods have been refined in the last one hundred years improving growth and detection of *Escherichia coli*. Standard techniques operate by selection against non-enteric bacteria (temperature, bile, dyes) and differentiation of *E. coli*. Nevertheless, *E. coli* represent a heterogeneous group. We are evaluating "field test kits" including one or more standard inhibitors along with some proprietary detection schemes for application in developing nations. Inhibition of *E. coli* with media constituents or techniques would of course represent "false negative" tests. In this investigation, we evaluated the growth of twenty *E coli* isolates, representing surface water, duck, chicken, cow, dog and human sources with standard inhibitors.
Twenty eight percent failed to grow at 46C on Hektoen Agar and TSA, however all grew at 43-44.5C. No isolates were inhibited by growth in pH 4 or pH 7 solutions of LB broth containing 0.5 – 4% Ox gal or Deoxycholate. Identical bile treatments utilizing the disk diffusion method with TSA yielded the same results. Twenty percent failed to grow at 4% NaCl + TSB; all failed at 8%.
Standard inhibitors (including 44.5C), did not affect the growth of these isolates. Salt and maximum temperature tolerance, however, indicated differences between these strains.

Co-Infections of Rocky Mountain Spotted Fever and Ehrlichiosis in American-English Crossbred Fox Hounds – A Case Study

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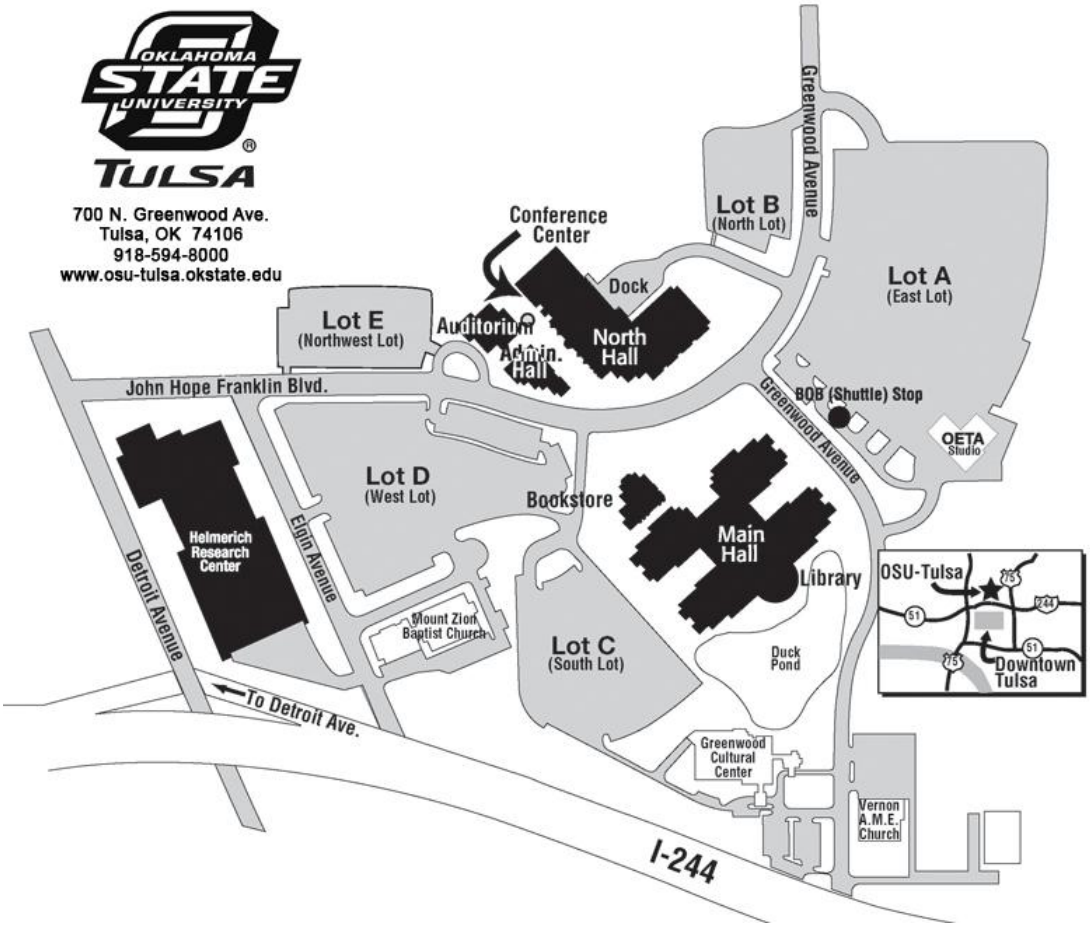
Medical Laboratory Science Department[1], Northeastern State University, Broken Arrow, OK;
Department of Biochemistry and Microbiology[2], and Forensic Sciences[3], Oklahoma State University-Center for Health Sciences, Tulsa, OK

Rocky Mountain spotted fever and Ehrlichiosis are tick borne diseases caused by *Rickettsia rickettsii* and *Ehrlichia canis*, respectively, that infect humans and animals. Infection with one of these bacteria can make an animal ill and co-infection makes the illness more severe. The purpose of this study was to evaluate a fox hunting kennel infected with Rocky Mountain spotted fever for co-infection with Ehrlichiosis. The kennel was infested with the Brown Dog tick, which can carry both *R. rickettsii* and *E. canis.* Thirty nine hounds had their blood drawn and tested for antibodies to both bacterial species using an indirect immunofluorescent antibody (IgG) assay. All hounds were retested three weeks later. Ten hounds showed either an increase in antibody titers or high antibody titers to both bacteria. Three of the ten infected hounds presented symptoms of the infections and were successfully treated with doxycycline. All hounds are currently well and show no signs of illness.

MAPS AND DIRECTIONS

OSU – Tulsa Campus
700 N. Greenwood Ave, Tulsa, OK 74106

Campus Map
http://www.osu-tulsa.okstate.edu/news/campusmap.php



Campus buildings

- T-MCB = **Main Hall** (Main Classroom Building)
- T-NCB = **North Hall** (North Classroom Building)
- T-HRC = **Helmerich Research Center**

FREE PARKING - Lot D or other lots

Directions to OSU-Tulsa

Turner Turnpike from Oklahoma City and west

Merge onto I-244 East via Exit 223A on the left toward downtown Tulsa. Take the Martin Luther King Jr. Blvd./Detroit Ave. exit on the left. Go to the second light, turn left onto Detroit Avenue. Move into the right lane. Watch for the campus on the right. Turn right on John Hope Franklin Boulevard.

SH-64/51 (Broken Arrow Expressway)

From the BA, exit at 75 North (Bartlesville). Move to the far left lane. Take the I-244 West exit. Then take the next exit, Martin Luther King Jr. Blvd./Detroit Ave. Turn right on Detroit Avenue. Move into the right lane. Watch for the campus on the right. Turn right on John Hope Franklin Boulevard.

I-244 (Crosstown or Martin Luther King Jr. Memorial Expressway)

Follow I-244 West to downtown Tulsa. When the highway splits, stay in the lane marked I-244 West (OKC). Take the next exit, Martin Luther King Jr. Blvd./Detroit Ave. Turn right on Detroit Avenue. Move into the right lane. Watch for the campus on the right. Turn right on John Hope Franklin Boulevard.

US-75 (from the north)

Follow US-75 South to I-244 West (OKC). Take the next exit, Martin Luther King Jr. Blvd./Detroit Ave. Turn right on Detroit Avenue. Move into the right lane. Watch for the campus on the right. Turn right on John Hope Franklin Boulevard.

From OSU in Stillwater, OK

Travel north on US-117 (Perkins Road) to the Cimarron Turnpike. Merge onto Cimarron Turnpike east toward Tulsa (includes two toll booths for a total of $1.25 each way). The Cimarron Turnpike becomes US-412 E. Take the I-244 E/US-412 exit on the left toward the L.L. Tisdale Pkwy/Joplin. Keep left to take US-412 E. Take the Martin Luther King Jr. Blvd./Detroit Ave. exit (Exit 6A) on the left. Turn left onto N. Detroit Ave. Turn right on John Hope Franklin Boulevard.

Travel from OSU-Tulsa to Holiday Inn to OSU Center for Health Sciences

A) OSU-Tulsa Bookstore 700 N Greenwood Ave Tulsa, OK 74106

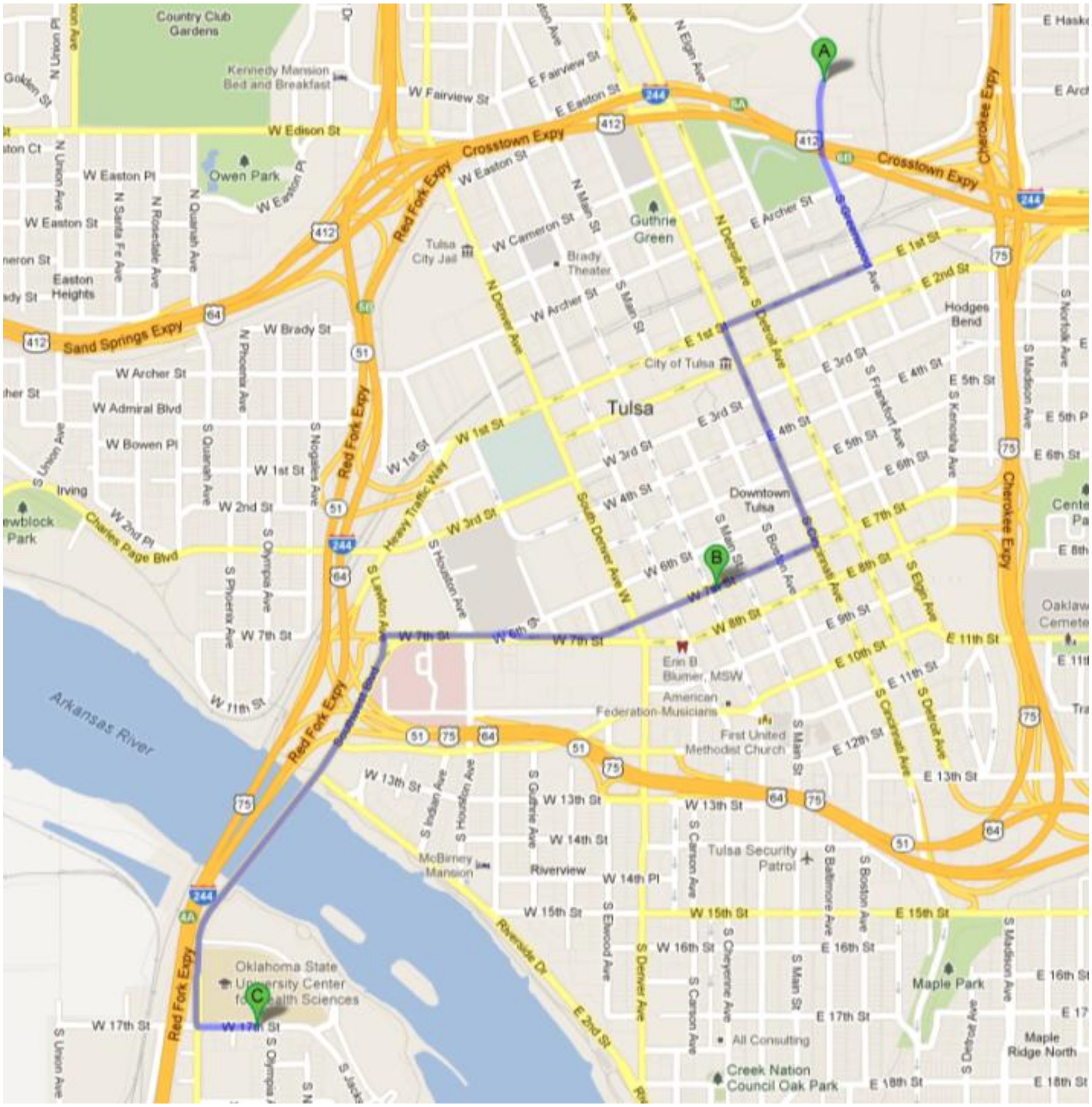
1. Head south on N Greenwood Ave toward E Cameron St 0.4 mi
2. Take the 3rd right onto E 1st St 0.3 mi
3. Turn left onto S Cincinnati Ave 0.4 mi
4. Turn right onto E 7th St Destination will be on the right 0.2 mi

B) Holiday Inn Tulsa City Center 17 W 7th St Tulsa, OK 74119

5. Head southwest on W 7th St toward South Boulder Ave W 0.6 mi
6. Turn left onto E 43rd St/S Lawton Ave/Southwest Blvd Continue to follow Southwest Blvd 0.8 mi
7. Turn left onto W 17th St Destination will be on the left 0.1 mi

C) OSU Center for Health Sciences, 1111 W 17th St Tulsa, OK 74107

Show on Google Maps

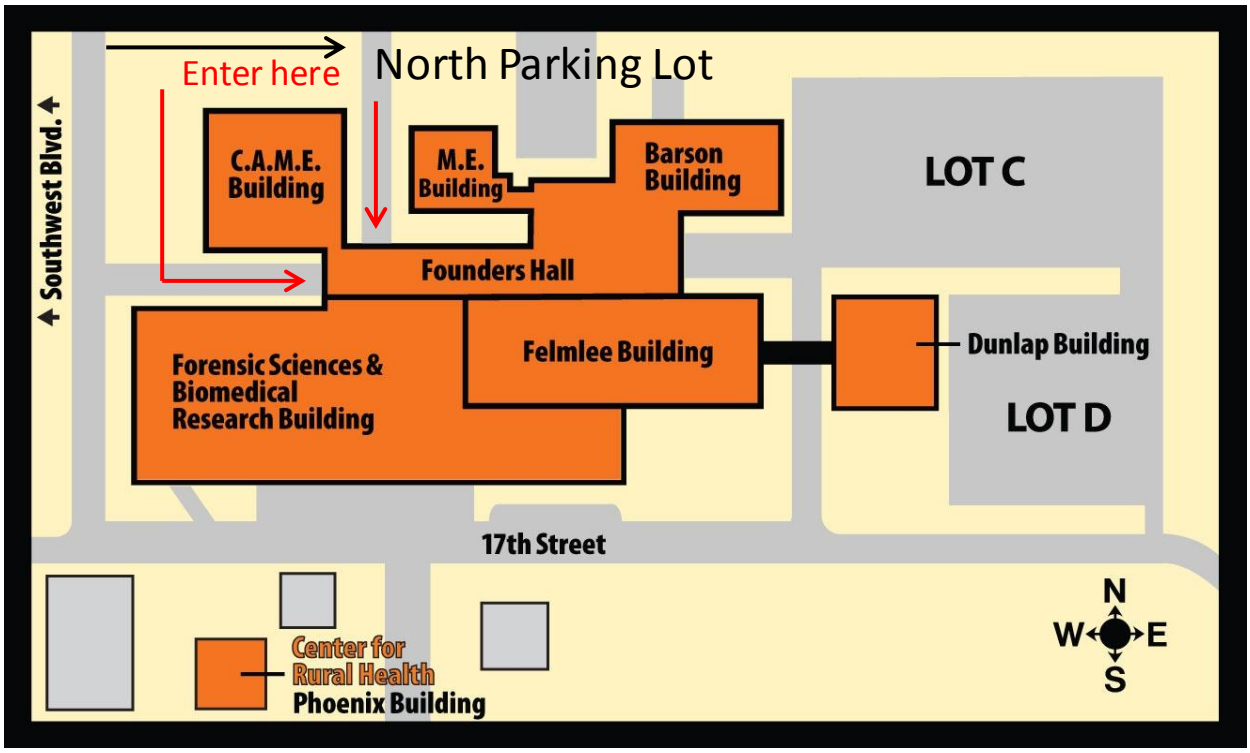


©Google

OSU Center for Health Sciences
1111 West 17th Street, Tulsa, OK 74107

Campus Map

http://www.healthsciences.okstate.edu/about/campusmap.php



PARKING: North Parking lot (North of C.A.M.E. and M.E. building) or Lot C, D

Directions from

Cimarron Turnpike

To OSU Center for Health Sciences:

- The Cimarron Turnpike (US 64, US 412) becomes the Keystone Expressway as you approach Tulsa from the west
- Exit on I-244 West (Oklahoma City)
- Once on I-244, immediately merge to the left before it becomes the downtown exit
- Continue on I-244
- As you approach the I-244 Arkansas River Bridge, merge right
- After you cross the river, take the first exit Seventeenth Street and Southwest Blvd (4A)
- At the bottom of the exit ramp, turn left (east) onto 17th Streeet
- Continue east through the stoplight
- The College is the tall building to the left
- Turn left into the first entrance

US 75/244 from the North

To OSU Center for Health Sciences:

- Going south on US 75/244, pass over North Peoria Avenue
- Exit I-244 West to Oklahoma City
- After crossing the river, exit on Seventeenth Street and Southwest Blvd. (4A)
- At the bottom of the exit ramp, turn left on 17th Street
- Go straight through the stoplight

- The College is the tall building to the left
- Turn left into the first entrance

US 75/244 from the South

To the OSU Center for Health Sciences:

- Proceed north on US 75
- Pass over I-44 and continue north (about 1 mile)
- Exit on Southwest Blvd. (3A)
- At the bottom of the ramp, turn left onto Southwest Blvd.
- Go to second light, turn right on 17th Street
- The College is the tall building to the left
- Turn left into the first entrance

West on 21st Street

To the OSU Center for Health Sciences:

- Head west on 21st Street and cross the 21st St. Arkansas River Bridge
- 21st Street becomes 23rd Street
- At the first stoplight, turn right (north) onto Jackson Ave.
- Jackson Ave. curves west and becomes 17th St.
- Look for the orange signs marking the Center parking lot on the right

Broken Arrow Expressway

To the OSU Center for Health Sciences:

- Travel west on the Broken Arrow Expressway (SH 51, US 64)
- Go past the exit for Houston Avenue
- Tulsa Regional Medical Center is on the right
- Exit left on I-244 West to Oklahoma City
- Immediately merge into the far right lane
- After crossing the river, exit on 17th St. and Southwest Blvd. (4A)
- At the bottom of the exit ramp, turn left (east) on 17th St.
- Continue east through the stoplight
- The College is the tall building to the left
- Turn left into the first entrance

Turner Turnpike/I-244 (Oklahoma City)

To the OSU Center for Health Sciences:

- After leaving the Turner Turnpike, proceed north toward Tulsa Downtown (left road of the intersection of I 44 and I 244) on I 244
- Exit on Southwest Blvd. (3A)
- Turn left onto Southwest Blvd.
- At the second stoplight, turn right onto 17th St.
- The College is the tall building to the left
- Turn left into the first entrance

To the OSU Health Care Center:

- After leaving the Turner Turnpike, proceed north toward Tulsa Downtown (left road of the intersection of I 44 and I 244) on I 244
- Exit on Southwest Blvd. (3A)
- Turn left onto Southwest Blvd
- The OSU Health Care Center is on the right