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**2012 Undergraduate Research Symposium Abstracts**

**GUT MICROBIOE DYSBIOSIS IN MICE WITH ALTERED INNATE IMMUNITY**


Type 1 diabetes (T1D) is an autoimmune disease resulting from the destruction of insulin-producing pancreatic beta cells. T1D can be prevented by invariant natural killer T cells (iNKT) in nonobese diabetic (NOD) mice. As activation of iNKT cells requires CD1d presentation, CD1d knockout mice lack the ability to activate iNKT cells, increasing the susceptibility of T1D. Additionally, an aberrant microbiome is believed to facilitate the onset of T1D. The link between CD1d and the intestinal microbiome is investigated here. NOD mice were separated into three distinct treatment groups — a NOD mice control, a gene knockout of CD1d, and treatment with KRN7000. DNA was extracted from large intestine and ileal stool samples. Paired-end, barcoded, 16S rRNA reads from the DNA samples were sequenced using Illumina. Results were analyzed for statistically significant differences between treatments using correlation tests in R and Rattle and a modified Wilcoxon rank test. Of the two treatments, CD1d knockout mice and KRN7000, the knockout mice showed more significant results compared to controls. The stool of the CD1d knockout mice showed a statistically significant percent variation in reads in six genera and their associated species. Of these genera, Helicobacter, Bifidobacterium, and Mucispirillum show correlations of 84.3%, 81.0%, and 85.8% respectively. The other genera, Allobaculum, Akkermansia, and Anaeroplama also showed significant differences between test and control with correlations each of 65%. The knockout of CD1d appears to increase bacteria that colonize the mucus layer and stimulate mucin production (Mucispirillum), while decreasing populations of mucin degrading bacteria (Akkermansia). The knockout also decreases a probiotic-like bacteria (Bifidobacterium) and increases Helicobacter, known to cause inflammation in the gut. These results suggest that a CD1d knockout has a dramatic effect on gut health, with the aberration leading to a possibility of a leaky gut followed by autoimmunity for type 1 diabetes.

**COEXPRESSION OF THE TATA-BINDING INITIATION FACTOR E AND ARCHAEOAL UBQUITIN-LIKE PROTEINS**

O. Olivar ez, N. L. He powit, H. V. Miranda, J. A. Maupin-Furlow; Department of Microbiology and Cell Science

Post-translational modification by small ubiquitin-like archaeal modifier proteins (SAMPs) expands the structural and functional diversity of the archaeal proteome. Sampylation is characterized by the formation of an isopeptide linkage between the C-terminal Gly of SAMP and α-amino group of Lys residues of protein substrates. One of the protein targets of sampylation in the haloarchaeon Haloferax volcanii is the TATA-binding initiation factor E (TbpE). SAMP-modification is speculated to dynamically modulate the properties of TbpE including half-life and intermolecular interactions. To understand the regulatory roles of SAMP on TbpE function, we cloned Flag-tagged SAMP genes in tandem with a gene encoding a TbpE-StrepII-tag fusion and expressed these genes from a constitutive P2 promoter in H. volcanii. Synthesis of the TbpE and SAMP proteins was readily detected by Western blot. SAMP-conjugated TbpE is detectable by α-FLAG and α-StrepII immunoblotting of StrepTactin column-purified TbpE. Current work is focused on mapping of conjugation sites that would eventually provide insight into whether sampylation positively or negatively promotes TbpE binding to the core promoter region of protein-coding genes. Hence, construction of the SAMP-TbpE tandem plasmids would further contribute in understanding transcription initiation complex assembly, which is a rate-limiting step of transcription activation.
THE PROTECTIVE ROLE OF SOCS1 AGAINST THE DEVELOPMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS
N. Robbins, J. Larkin Department of Microbiology and Cell Science

Systemic Lupus Erythematosus (SLE) is a T cell mediated autoimmune disorder that appears with symptoms that could be mild or as severe to cause death. Studies have shown that effector T lymphocytes play a role in the development and pathogenesis of lupus. Furthermore, upon observation of F1 generation New Zealand Black/White mice (the classic models for SLE), it was found that these mice are partially deficient in SOCS1. Also, SOCS1 heterozygous (SOCS1 deficient) mice present with lupus-like symptoms eight months after birth. This suggests that SOCS1 may play an integral role in the development of SLE. In the present study, to determine the role of SOCS1 in T cell differentiation, experiments were performed on SOCS1+/+ and SOCS1+-/- mice, focusing specifically on Foxp3+ regulatory T cells (Tregs) and Th17 lymphocytes. We observed that despite equal proliferation between cells from SOCS1-/- and SOCS1+-/- mice, SOCS1 deficient mice produce significantly less IL17A under Th17 inducing conditions. Notably, under the same conditions, SOCS1+/- mice show higher concentrations of SOCS1+/+ mice, SOCS1 deficient mice inhibit the growth of Th17, even under Th17 inducing conditions. The dysregulation of IFN-γ production may be leading to the chronic autoimmune response and progression of SLE. The correlation between SOCS1 deficiency and improper T cell differentiation in these lupus prone mice may provide a basis for new lupus therapy via restoration of the SOCS1 protein.

CHARACTERIZATION OF NITRIC OXIDE SYNTHASE (NOS) EXPRESSION AND ITS POTENTIAL CONTRIBUTION TO VIRULENCE IN STAPHYLOCOCCUS AUREUS
N. Bhut, Nakul, B. Black, A. Sapp, K. C. Rice
Department of Microbiology and Cell Science

Nitric Oxide (NO) is thought to play an important role in Staphylococcus aureus cell physiology and oxidative stress tolerance. S. aureus NO synthase, a homologue to eukaryotic NOS enzymes, is encoded by a nos gene present in all sequenced S. aureus genomes. Previous expression studies in our lab demonstrated that nos is co-transcribed with a downstream prephenate dehydratase (pdt) gene, and RNA levels are increased during low-oxygen growth. However, virtually nothing is known about the environmental and genetic regulation of nos. To this end, the 500 bp sequence upstream to the putative nos ribosomal binding site was amplified by PCR, and cloned upstream of green fluorescent protein (GFP) to generate a transcriptional GFP reporter. A positive GFP control using the highly-active ciaA promoter, and negative control (promoterless GFP) were also created. GFP fluorescence during planktonic growth under aerobic and low-oxygen conditions was assessed at 3, 6, 12, and 24-hour time points. Flow cytometry was also used to quantify the cell population producing GFP. The results indicated that on average, 96% of the cell population expressed the ciaA-GFP plasmid in both low and high oxygen environments. Expression of nos-GFP was significantly lower in both testing conditions, whereby nos-GFP expression occurred in only 10% of the total cell population. This result, combined with lack of strong -10 and -35 elements in the putative nos promoter sequence, suggests that nos promoter activity is highly regulated and only occurs in a subpopulation. A modified C. elegans infection model was also utilized to examine the contribution of nos to S. aureus virulence. Preliminary investigations have indicated that nos and nos-pdt mutants showed attenuated worm killing, suggesting that these genes may play an important role in survival in vivo. On-going and future research efforts are aimed at identifying genetic regulators of S. aureus nos expression.

IDENTIFICATION OF BACTERIAL BIOCONTROL AGENTS IN BIOLOGICAL SAMPLES
M. Ohanesian, M. Vidales, D. Brackett, J. McLean, M. Oli
Department of Microbiology and Cell Science

Many microorganisms in nature produce secondary metabolites in response to various environmental conditions. Bacteria that participate in the decomposition of organic matter are known to produce chemical substances or enzymes that destabilize certain cell components, aiding in the degradation or inhibition of growth of other cells. In order to isolate and identify bacterial species with the potential to provide new antibiotics that could be harvested and utilized on a wide-scale, we collected various samples from decaying wood, soil, and water. From these samples, we were able to isolate four species that produce prospective antibacterials. When the filter-sterilized supernatants were applied to Staphylococcus aureus and Escherichia coli in an antibiogram assay, differential inhibition of growth was observed. To identify each microorganism, DNA was extracted and 16S rRNA gene analysis was conducted. Blast results of the sequences showed that products from two strains of Pseudomonas inhibited the growth of S. aureus more so than the growth of E.coli. The other bacteria identified as potential antibiotic producers include Bacillus and Rhodococcus. Further testing of the metabolites, subsequent isolation and characterization of the active compound could potentially to the development of novel antibiotics.

THE 2'-5' RNA LIGASE LIGT IS INVOLVED IN STRESS RESPONSE
D. Baxter, P. Thiaville, J. Thiaville, V. de Crécy-Lagard
Department of Microbiology and Cell Science

Nucleotide damage repair is essential for survival of the cell. Damage to nucleotides can occur via oxidation or breakage caused by free radicals or gamma irradiation. Damage to RNAs can lead to several phenotypes including: stalled translation from tRNA damage and failure to adapt to stress due to small RNA damage. Cina of B. subtilis has been implicated in nucleotide repair, and a homolog of cina was identified in Deinococcus radiodurans capable of repairing irradiation-induced DNA damage in E. coli ΔrecA1. The aim of this research is to determine which genes are responsible for repairing RNA damage due to various stresses. We used comparative genomics analysis and data mining technologies to predict sfsA, ligt, yadB, kptA, and ybjD could be involved in repair of RNA damage. We tested individual mutations of each gene in E. coli by stressing the cells through growth at either high temperatures or presence of hydrogen peroxide. Of the mutants tested, AligT failed to grow at 42°C and was more sensitive to hydrogen peroxide. ligt is annotated as a 2'-5' RNA ligase (Arn and Abelson, JBC. 1996), but the cellular role has not been defined. It is known that in vitro the LigT protein repairs tRNA halves; however, our own analysis of tRNA from AligT did not indicate an increase in cellular tRNA halves. Future work will continue to identify the biological role of ligt and its role in response to stress.
Type 1 diabetes (T1D) is an autoimmune disease, which causes the destruction of insulin-producing β cells in the pancreas. Stimulation through toll-like receptors (TLRs) on leukocytes initiates the innate immune response, resulting in production of pro-inflammatory cytokines, such as IL-6, and anti-inflammatory cytokines, such as IL-10. The single nucleotide polymorphism (SNP) rs5979785 (T→C) near the TLR8 gene on the X chromosome has been shown to decrease TLR8 gene expression, and is protective in T1D. We hypothesized that patients with T1D would produce more pro-inflammatory cytokines when stimulated with TLR agonists, and that the rs5979785 genotype would affect TLR7/8-induced cytokine production. To test this, we diluted peripheral blood from T1D patients (n = 32) and controls (n = 23) in media and stimulated in vitro with 10 ng/mL LPS (TLR4 agonist) or 1 ug/mL R848 (synthetic TLR7/8 agonist). After a 24-hour incubation, supernatants were collected and analyzed for IL-6 and IL-10 production by ELISA. rs5979785 genotype was determined by Taqman real-time PCR. We found no significant difference in IL-10 or IL-6 production between patients and controls when stimulated with LPS or R848. However, rs5979785 genotype did modify cytokine production in R848-stimulated, but not LPS-stimulated, cells. Among the combined T1D and control cohorts, there were 32 individuals with TT or 0T genotype (17 female, 15 male), 13 females with TC, and 8 individuals with CC or 0C (1 female, 7 male). A student’s t test revealed that there were unequal variances in IL-6 production between TT+0T, TC, and CC+0C groups (P = 0.0044). There was also a slight unequal variance in IL-10 production between the groups (P = 0.0423). These results show a specific effect of TLR8 SNP rs5979785 on TLR7/8-induced cytokine production. Decreased pro-inflammatory cytokine production associated with the allele may be the mechanism of protection in T1D.
Abstracts for poster presentations

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HIV Made Simple
E. Pierre-Paul, M. Oli; Department of Microbiology and Cell Science

Since the availability of highly active antiretroviral therapy, the number of HIV-infected children surviving childhood is increasing. HIV-infected children are growing curious as they become more aware of the demands of their health. They are asking questions and most caregivers and parents are providing inaccurate or elusive information. Research indicates that strong parental relationships and full disclosure of HIV status by caregivers to children are associated with good adherence. Yet, parents are still concerned about the impact of disclosure on the mental and emotional health of the child. While many studies have defined the importance of disclosure, seldom has a study provide ways to assist parents and caregivers in the process of disclosing and educating children about their diagnosis. This study was conducted to provide parents and caregivers with sound, fun, and interactive ways to deal with the problem of proper disclosure and education of HIV to children. It is also to educate children that are not infected about the disease to clear the stigmas that may plague infected children. There were 16 HIV-infected individuals and 15 uninfected involved in the study. Eight of the infected persons were children aged 4 to 10 years that were not yet aware of their diagnosis, and the remaining 8 were teens aged 13 to 17 years who were informed in the past with little or no educational or moral support. All groups were exposed to and engaged in all activities developed. The uninfected children behaviors and knowledge about HIV were assessed prior to and after being educated. The teens were interviewed and shared their insight on how these tools would have either benefited or affected them during and after their personal disclosures. Finally for those infected, observations were based on their behaviors towards treatment, themselves, and HIV before and after the disclosure and teaching. This study will highlight the benefits and need for the development of fun and interactive educational tools and models to assist caregivers, parents, and educators in providing accurate and sound information to children when discussing HIV.

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ASYMPTOMATIC CARRIERS OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS - HORSE AND OWNER
L. Beveridge, E. Dammers, M. Oli; Departments of Animal Sciences and Microbiology and Cell Science

Over the last years it has been recognized that pets and livestock are a reservoir, asymptomatic carriers, of methicillin-resistant Staphylococcus aureus (MRSA). A 2011 case report highlighted an incidence of MRSA transmission from a Friesian foal to a young girl. This article sparked our interest to determine if (1) horses in Alachua County are MRSA carriers, (2) if there is a possible correlation of hospital stay of the horses and the presence of MRSA and (3) if there is a correlation of MRSA occurrence in horses and their owners. Four privately owned horses were screened for MRSA by taking nasal swabs from each horse and inoculating CHROMagar MRSA II plates. Similarly, nasal swabs were examined from the horses’ owners. MRSA positive colonies were further characterized and confirmed as oxacillin resistant, coagulase positive, S. aureus strains. 16S rRNA gene sequence analysis was conducted to confirm MRSA positive isolates. The medical history of the horses was examined, hospital stay was noted and veterinarians were interviewed for their experience with MRSA in horse in Alachua County. We determined that one horse out of the ten tested was MRSA positive. The MRSA negative horses did have positive growth for multiple other methicillin resistant microbes, but through further testing (gram stain, growth on blood agar plate, Gideon database) were excluded to be MRSA. The horse that was found MRSA positive had a recent stay at an animal hospital. The sample collected from the owner’s nares also contained MRSA. We are currently in the process to compare 16S rRNA gene sequences of the horse and human isolate, perform further tests to determine if horse and owner are carriers of the same subspecies.
Bbcα1, was identified and characterized. The role of the caelestin protein was investigated via transmission electron microscopy. Germinated conidia and conidia were grown in various media over a time course. Targeted gene inactivation of Bbcα1 did not result in any noticeable effects on lipid droplet formation, nor were any lipid substrate growth defects apparent. In contrast to the wild-type parent, however, production of the multilamellar vesicle bodies was constitutive in the ABbcα1 mutant, and accumulation of these vesicles was noted in the mutant. Insect bioassays using the Greater Wax Moth, Galleria mellonella, revealed reduced virulence in the mutant as compared to the wild-type parent. Our data indicate a novel function for caelestins in the trafficking and/or degradation or turnover of the newly described multilamellar vesicles.

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CESA 10 GENE IN ENERGY CANE
K. Tran, J. Wang; Department of Agronomy

Biofuel are gaining in popularity from the science community and public due to fuel prices, the movement to go green, and to find an alternative fuel. One gene in peculiar that would help in the production of biofuel is the CesA gene to increase a plant’s biomass which increases the amount of biofuel. The plant’s cell wall is primarily made up of cellulose, hemicellulose, and lignin. The CesA, consists of CesA 1-10, genes are very important genes that form either the primary or secondary cell wall thickening, known in Arabidopsis shoot and Cotton Seed Trichomes. A homolog gene of the CesA 10 gene will be isolated in the Energy Cane (US09-1137) using the known sequence of the CesA 10 gene from the closely related family of Sorghum Bicolor. After retrieving the Energy Cane and extracting the DNA, a BLAST search of the Sorghum Bicolor gene resulted in the specific sequence of the CesA 10 gene and primers were made. After running a PCR, Sanger Sequencing helped to find the exact sequence of the gene. Further research must be formed to verify that this is indeed the CesA 10 gene in Energy Cane.

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LACTOBACILLUS JOHNSONII DECREASES INTESTINAL IDO CONTENT AND ACTIVITY
L. Bujitova, E. Cameron, R. Valladares, C. Gonzalez; Department: Microbiology and Cell Science

Interactions between intestinal commensal microbes and their hosts play a vital role in the proper development of the intestine as well as in immunological development. The enzyme indoleamine 2,3-dioxygenase (IDO) is highly expressed along the intestinal tract, where it catalyzes the rate-limiting step of tryptophan catabolism via the kynurenine pathway. Previous studies have shown that host expression of IDO is highly dependent on the microbial colonization of the intestine. IDO also plays a key role in T cell differentiation and host neurological status. Previous work by our lab has shown that the commensal bacterium Lactobacillus johnsonii strain N6.2 (L. johnsonii) decreases IDO expression in the host ileum in vivo. Furthermore, L. johnsonii cell-free supernatant can inhibit recombinant IDO enzymatic activity in vitro. We hypothesized that the presence of L. johnsonii decreases the amount of IDO protein and enzymatic activity in the host intestine. We tested this hypothesis using two models. First, we analyzed the IDO protein content of intestinal tissue and mesenteric lymph node lysates of 60-day-old rats fed 108 CFU/day of L. johnsonii. Secondly, we quantified the IDO activity of IFNγ treated intestinal epithelial cells (IEC) exposed to L. johnsonii cell-free supernatant. The total IDO content of normalized tissue lysates were compared using densitometry. The IDO content of the ileum and mesenteric lymph nodes of L. johnsonii treated rats was significantly lower than age-matched PBS-fed controls. The kynurenine/tryptophan (product:substrate) ratio of IEC supernatants treated with L. johnsonii cell-free supernatants was significantly lower than IFNγ treated positive controls. These results suggest that L. johnsonii produces a soluble mediator that decreases IDO expression and activity in host cells. Currently, we are investigating the identity of this mediator and the consequences of decreased IDO activity in host tissues.

The purpose of this study is to identify circulating tumor cells (CTC) in the blood of patients with glioblastoma (GB) multiforme, the deadliest type of brain cancer. Currently, GB is thought not to metastasize out of the brain, but there are reports of systemic metastasis of GB in the lungs, lymph nodes, skin, and liver. The current way to monitor GB status is through indirect imaging (MRI) because repeat resection involves significant morbidity, time, cost, and does not result in improved survival for most patients. The inability of either indirect radiographic or clinical monitoring to provide an accurate tumor status together with an absence of cellular or molecular data on how the tumor is responding to treatment and changing over time creates a need for direct characterization. Such an approach would not only benefit treatment decisions made today but also provide valuable information on tumor response and guide appropriate experimental therapeutics in clinical trials with “real time” data. The CTCs will be captured in the peripheral blood through an assay developed to find GBM cells in a 1:1,000,000 ratio with WBCs. Negative sorting by way of flow cytometry of WBCs using a specific dual antibody system will be used concurrently with a positive sorting system of GB cells that express high levels of antigen not found on WBCs. The assay for specific recognition of GB cells in the peripheral blood is what will be ultimately developed and tested through this project.

DIFFERENCES IN PROTEOBACTERIA AND BACTEROIDETES POPULATIONS CORRELATED WITH MODE OF DELIVERY IN PREMATURE INFANTS
K. Rechcigl, M. King, A. Ardisone, R. Margas-Torrazza, J. Neu, E. Triplett; Department of Microbiology and Cell Science

There is abundant evidence that particular microbial groups are correlated with various disease states, and numerous studies are attempting to define a “healthy” human microbiome. The human intestinal microbiome can be influenced by a variety of factors. Of particular interest are factors that influence initial microbial establishment as this has potential implications with early immune development. Correlations between mode of delivery and intestinal microbiota have been ambiguous. The aim of this study is to investigate differences in the microbial population of 11 cesarean and 8 vaginally delivered premature infants. 16S rRNA gene Illumina sequencing was performed on meconium samples from premature infants with an average gestational age of 29.6 weeks. Using chi-square statistical analysis, Proteobacteria were significantly more abundant in cesarean-delivered infants (α = 0.01; p = 0.00565); whereas Bacteroidetes were significantly more abundant in vaginal-delivered infants (α = 0.01; p = 0.00156). These differences were observed at deeper taxonomic levels as well, and these differences greatly diminish after the first week of life. Contrary to other studies, no correlation with gestational age was found amongst meconium samples in this study, and correlations between microbial abundances with other metadata were examined. Therefore, according to this study, a significant difference in the founding microbial population of cesarean and vaginal-delivered premature infants exists. Such early disparities may provide incite towards long-term implications of an individual’s health and development.
4 LOCAL ADIPONECTIN PRODUCTION IN SKELETAL MUSCLE RESISTANCE ARTERIES: EFFECTS OF EXERCISE AND SHEAR STRESS
G. Sapp, B. Chen, A. Garovich, J. Muller-Delp
Department of Physiology and Functional Genomics

Adiponectin is an adipose-derived secretory protein with antiinflammatory and anti-atherosclerotic effects in the endothelium and vascular smooth muscle. Paradoxically, despite being secreted from adipose tissue, plasma adiponectin correlates inversely with obesity and obesity-associated cardiovascular disease. The purpose of the current study was to determine whether the endothelium of resistance arteries is a local source of adiponectin. We also investigated the possibility that exercise training upregulates adiponectin in rat skeletal muscle resistance arteries. Real-time qRT-PCR was used to assess mRNA for adiponectin in both endothelium-intact and -denuded arteries, and in cultured endothelial cells exposed to shear stress. Adiponectin mRNA was also assessed in skeletal muscle resistance arteries isolated from sedentary and exercise trained rats. Adiponectin mRNA was detected in both intact and denuded arteries. Shear stress increased adiponectin mRNA in cultured endothelial cells, and exercise training increased adiponectin expression in skeletal muscle resistance arteries. These data indicate that both the endothelium and the vascular smooth muscle of skeletal muscle resistance arteries are local sources of adiponectin. Exercise training increases adiponectin expression in skeletal muscle resistance arteries, possibly through a shear stress-sensitive mechanism.

5 THE ROLE OF SALMONELLA PATHOGENICITY ISLANDS IN THE INTERACTIONS WITH TOMATOES
A. Aruca, M. Teplitski
Department of Microbiology and Cell Science

In today’s food market, there is a need to understand how human pathogens contaminate minimally processed foods and whether pathogens in minimally processed foods are a danger to our food supply. Furthermore, interactions of human pathogens with alternate hosts (like plants) present an interesting evolutionary and ecological challenge: do human pathogens rely on the same genes to persist within plants as they use to infect animals? Using competitive index (calculated based on the co-infections of the wild type Salmonella and its mutants defective in SPI-3, SPI-4, SPI-5, STM4257, STM4258 and STM4259), we compared the role of Salmonella virulence genes in persistence within tomatoes. This is important to show how mutant strains can appear and become more virulent against wild type and how this may prove to be problematic in the future. Kanamycin-resistant Salmonella enterica sv Typhimurium mutants lacking SPI-3, SPI-4 and SPI-5 were infected into red ripe tomatoes with equal amounts of the wild type cells. Infected tomatoes were incubated at 20°C, blended in phosphate-buffered saline and plated onto XLD medium to recover Salmonella. Salmonella colonies were patched onto LB with or without kanamycin to distinguish the mutants from the wild type. By comparing the two, we can see how the mutant strains compete against the wild type strains and which specific pathogenicity islands, or genetic deletions, is superior or inferior. SPI-5 mutants were as competitive as the wild type strain, SPI-4 is capable of persisting in tomatoes better than the wild type, while SPI-3 was slightly less persistent. It is important to study these virulence factors to have a between understanding of each mutant strain’s effect compared to the wild type and how this will impact us in the future.

33 Characterization of xyn10A from Xanthomonas axonopodis pv. citri and its contribution to the depolymerization of 4-O-methylglucuronoxylan
R. Lipworth, M. S. Rhee, V. Chow, G. J. D. Rice, J. Preston
Department of Microbiology and Cell Science

Plant pathogenic species of Xanthomonas produce glycoside hydrolases that catalyze the depolymerization of cell wall polysaccharides derived from host tissues, and the participation of these enzymes in the deconstruction of cell walls may contribute to plant disease. The sequenced genome of Xanthomonas axonopodis pv. citri (XAC), the bacterial agent causing citrus canker, has identified operons including xyn10A, xyn10B, and xyn10C genes encoding three endoxygenases of glycoside hydrolase family 10 (GH10) and an aga67 gene encoding a GH67 α-glucuronidase. Reombinant enzymes encoded by the xyn10A and aga67 genes have been partially characterized with respect to products formed from synthetic as well as complex plant-derived substrates, defining their respective contributions to the depolymerization of 4-O-methylglucuronoxylan. The synergistic action of these enzymes in the depolymerization of methylglucuronoxylans supports a role in the pathogenic process.

34 CONJUGATION OF BACTERIAL THIS AND HUMAN UBQUITIN IN AN ARCHAEAAL HOST
A. Berganini, N. Hepovit, H. Miranda, J. A. Maupin-Furlow
Department of Microbiology and Cell Science

Ubiquitylation, an E1-dependent conjugation of ubiquitin to target protein substrates, is a universal key regulatory mechanism in various cellular processes in eukaryotes. In bacteria, the ubiquitin-like protein ThiS, is not involved in protein modification but rather it serves as a sulfur donor in a pathway leading to the synthesis of thiamin, a process which is ThiF-dependent. In archaea, the small ubiquitin-like protein SAMP2 is capable of conjugation and sulfur transfer (during thiamine biosynthesis) when activated by an E-1/ThiF-like enzyme called UbaA, thereby linking the two divergent biochemical pathways, protein conjugation and sulfur transfer. To further substantiate our previous results about the role of UbaA in both protein conjugation and sulfur transfer, we introduce in trans the human Flag-ubiquitin and E. coli Flag-ThiS in the haloflexus Haloferax volcanii Δxamp1Δxamp2αxamp3. Surprisingly, in the presence of DMSO, both ubiquitin and ThiS can form covalent-linked conjugates with archaeal proteins in a UbaA-dependent manner, suggesting that UbaA can activate ubiquitin and ThiS similarly to its eukaryotic and bacterial analogs, E1 and ThiF, respectively. Deletion experiment shows that the C-terminal GlyGly of ThiS is crucial for its conjugation, which is speculated to be important in its adenylation by UbaA prior to being conjugated to its protein substrate. Furthermore, current efforts are underway to identify the archaeal protein substrates for ubiquitylation and ThiS-conjugation. Whether the exogenous ThiS can replace the role of SAMP2 in thiamin biosynthesis still remains to be determined.

35 INVOLVEMENT OF A CALEOSIN IN THE FORMATION OF A NOVEL LIPID-INDUCED MULTILAMELLAR VESICLE BODY IN THE ENTOMOPATHOGENIC FILAMENTOUS FUNGUS, BEAUVIERIA BASSIANA
R. Kudia, Y. Fan, N. O. Keyhani; Department of Microbiology and Cell Science

Eukaryotic cells accumulate lipids in membrane encased droplets. The entomopathogenic fungus, Beauveria bassiana, has a parasitic life cycle where infection initiates via attachment of its spores to the epicuticle or wax layer of target insects, degrading and assimilating host surface hydrocarbons, ultimately penetrating the host’s epicuticle. Although, B. bassiana produces lipid droplets, we report the biogenesis of a novel multilamellar vesicular body, which accumulate in fungal conidia grown on various lipids. Caleosins are a group of plant and fungal proteins that are thought to be minor components of the proteinaceous coat of lipid droplets. The gene for a B. bassiana caleosin homolog,
The purpose of the project was to identify chemical ligands that modulate the activity of selected Candidatus Liberibacter asiaticus (CLas) transcriptional regulators. CLas is an unculturable organism that causes Huanglongbing disease in citrus. The aim of this project is to identify ligands that may disrupt the activities of transcriptional regulators, and thus may also impair the physiological activities of this bacterium in citrus plants. Due to its reduced genome, CLas has only a few transcriptional regulators, and those selected for this study were CLIBASIA_00835, CLIBASIA_03370, and CLIBASIA_01180. The specific goals were to clone, express and purify CLIBASIA_03370 and CLIBASIA_00835 using Sinorhizobium meliloti as an expression host. The rationale is that by using a host that is phylogenetically related to CLas the solubility of the proteins would be improved, compared to the low expression and solubility previously obtained in Escherichia coli. Preliminary data identified five chemicals that modified the thermostability of purified CLIBASIA_01180. Thus, the second specific goal was to validate the effects of these chemicals in vivo that were found to decrease the DNA-binding activity of CLIBASIA_01180 in vitro. The results obtained indicate that the CLas genes can be expressed in S. meliloti. The CLIBASIA_00835, as well as CLIBASIA_03370, were expressed and partially purified. Additionally, the preliminary screening showed that some of the chemicals affect the in vivo activity of an ortholog of CLIBASIA_01180 in S. meliloti.

As a global regulator of gene expression, CsrA (carbon storage regulator) of Escherichia coli is a member of a family of proteins that posttranscriptionally regulate the translation and/or stability of numerous mRNAs. Riboswitches, cis-acting RNA elements, located in the untranslated segments of mRNAs, can serve to regulate translation initiation or translation initiation in response to ligand binding. Previous studies identified an RNA motif that is highly conserved in bacteria and is located upstream of genes that encode for enzymes involved in molybdenum cofactor (Moco) biosynthesis and proteins that utilize Moco as a coenzyme. The 5' untranslated leader of the moaABCDE operon of E. coli contains one of these motifs. This operon is required for Moco synthesis. Its untranslated leader forms a complex structure that is believed to be a riboswitch that senses Moco levels in the cell by binding to this cofactor. Studies using RNA gel shifts and footprinting with Fe-BABE conjugated CsrA protein showed that CsrA binds specifically to the moaA 5'UTR mRNA leader at two distinct regions. Here, we tested moaA-lacZ and lacUV5moaA-lacZ gene fusions for expression in csrA WT and isogenic csrA mutant strains by performing β-galactosidase assays at various time points after harvesting cells. Expression of these moaA fusions was substantially greater in the csrA WT strain background. We conclude that CsrA activates expression of the moaA gene. Our studies are consistent with a model in which the moaABCDE leader forms a riboswitch that interacts with two different regulatory factors, Moco and the CsrA protein, to control moaA expression in response to the availability of Moco and products of carbon metabolism, respectively.

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this experiment, we used a case/control design nested in a prospective cohort study of preterm infants delivered at a gestational age of less than 32 weeks and a birth weight of less than 1250 grams. Stool samples from nine case subjects were matched to those from eighteen controls, and samples two weeks before, one week before, and during the week of diagnosis were analyzed. After isolating community DNA from feces DNA isolation we used DGGE analysis, qPCR, and 16S rRNA sequencing to determine microbiota diversity and composition. DGGE analysis showed a general trend towards lower microbiota diversity in sepsis cases compared to our control subjects at two weeks before diagnosis but not one week before or during the week of diagnosis. We obtained 180845 16S rRNA sequences with an average length of 474 nucleotides, which were categorized into Operational Taxonomic Units (OTU) using ESPRIT-tree. We noticed multiple OTU’s that were commonly found in control subjects but lacked in cases. A proteobacterial bloom was observed during the week of diagnosis in cases, while an opposite trend was observed in controls. Real time PCR showed no Bifidobacterium in cases but almost half of control samples showed high levels of this bacterium, which is thought to be essential for microbiota development in infants. These results support our hypothesis that distortions of microbiota are associated with late onset sepsis.

9 INVESTIGATING THE ROLE OF CELL-TO-CELL SIGNALING IN THE PATHOGENESIS OF A POLYMICROBIAL YELLOW BAND DISEASE CONSORTIUM
R. Camacho, W. Zaragoza, M. Teplitski
Department of Soil and Water Science

Corals are holobionts; an obligate symbiosis of marine invertebrates, photosynthetic zooxanthellae, bacteria, and fungi. They form large ecosystems that act as reservoirs of ecological diversity. Corals suffer from diseases caused by polymicrobial consortia of opportunistic pathogens. Yellow Band Disease (YBD), one of these so-called polymicrobial consortiums, is responsible for reef degradation in the Caribbean. Cell-to-cell signaling, or quorum sensing, has been shown to be important during polymicrobial infections of higher organisms. The role of this intracellular communication during polymicrobial invasions of opportunistic pathogens is not fully understood. In this study we are investigating the role of acyl-homoserine lactones (AHLs) in the pathogenesis and virulence of YBD during the infection of a marine holobiont. The marine invertebrate Aiptasia pallida, a holobiont closely related to reef building polyps, was used for the investigation along with a laboratory-generated consortium of Vibrio spp., identified as potential pathogenic members of the YBD consortium. Polyps infected with the YBD consortium exhibited tissue necrosis and dose-dependent death within a week. The LD50 was determined to be 7.4 x 105 cfu per mL. Post-assay recovery yielded Vibrio isolates from the YBD consortium. Extracts of these Vibrio isolates were assayed for AHL production using an Agrobacterium reporter. Initial results show that two of the four isolates used in the minimal YBD consortium produce AHLs. Further assays will be done to isolate extracellular signals and characterization will be carried out with reverse-phase chromatography and synthetic in situ AHL reporters. Finally, AHL production will be disrupted in the YBD consortium and infections carried out to determine the affect, if any, of signaling disruption on the virulence of the consortium.

10 IDENTIFYING THE PREVALENCE OF NOSOCOMIAL INFECION causING AGENTS IN NON-ICU’S VERSUS ICU’S WHILE DEMONSTRATING THE INTRINSIC SANITIZING PROPERTIES OF COPPER ALLOY “TOUCH SURFACES”
F. Parks, C. Ho, L. Stewart, E. Cova, A. Tesfa
Department of Microbiology and Cell Science

Nosocomial infections are a cause of high mortality in many hospitals worldwide. These nosocomial infections can lead to pneumonia, urinary tract infections and even sepsis. Greater antibiotic resistance is spreading amongst the Gram-negative bacteria to the non-hospitalized. Those patients

converted to DNA. Changes in gene expression from omental and subcutaneous fat will be tested by SYBR Green Real Time RT-PCR. Among the many genes assessed, a few fat-related genes have shown approximate thousand-fold increases in expression in correlation with M-T7 treatment. Future plans involve testing these genes in mice treated with differing mutants of the M-T7 protein.

29 VIRULENCE OF PRIMARY ISOLATES OF COMMENSAL STAPHYLOCOCCUS EPIDERMIDIS IN GALLERIA MELLONELLA
A. Ewald, A. Youngs, M. Oil; Department of Microbiology and Cell Science

Staphylococcus epidermidis is an “accidental” pathogen that causes many problems in the public health system as opportunistic pathogen, present in abundance as commensal on the human skin. Research has been done to find genes that regulate virulence in S. epidermidis, but skepticism remains as to the specific mechanism of its virulence. The goal of our research was to determine if different primary isolates of S. epidermidis were virulent and showed different degrees of virulence in Galleria mellonella (greater wax worm). S. epidermidis isolates from the nasal flora of several individuals in the lab were isolated on Mannitol Salt agar plates and identified via Gram stain and 16S rRNA gene sequence analysis. Each strain was grown overnight in Tryptic Soy Broth enumerated and diluted to 107 CFU/mL with PBS before being injected into the last proleg of cooled wax worms. Controls included injection only, PBS only, and a positive control (S. aureus). Each group of 6 worms was incubated in a petri dish with food at room temperature. Melanization and movement upon touch were monitored daily for 6 days. Preliminary results showed similar virulence of S. epidermidis and S. aureus strains, while negative controls showed no signs of virulence or injury due to injection. Using the wax worm model to study host pathogen interaction and S. epidermidis virulence factors can improve our understanding of how S. epidermidis becomes pathogenic.

30 ENGINEERING THE CUTICLE FOR DROUGHT RESISTANCE
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Like all organisms, plants depend on water for survival. During drought conditions cuticular transpiration becomes a major factor that determines plant survival. One of the major defensive strategies of plants is to reduce stomatal apertures to prevent water loss. Once the stoma is closed the life of plant is dependent on the permeability of the cuticle. Engineering alterations on stoma cuticular level should have an impact on minimizing transpirational flux under drought conditions. By altering only plant epidermal thickness cell gene expression, we should expect to exert minimal deleterious effect on growth during non-water stress period. We will attempt to engineer drought resistance in plants by up-regulating the expression of genes involved in both cuticle synthesis and cuticular x-linking in epidermal cells and look for alterations that decelerate water loss. Initial experiments will be conducted in the model plant, Arabidopsis. Two promoters: Cer6 and At11 promoter have been cloned into p. Cambia 1305.1 where they will drive the expression of a GUS marker. These constructs have been transformed into Agrobacterium and the Agrobacterium transformants were used to generate transgenic plants. Transgenic plants will be stained for GUS expression. Currently the selection of transgenic plants is underway. We believe that cuticular modification should protect plants during drought periods. The development of strategies to modify and protect plants from water loss is important due to increasing worldwide drought conditions.
ADE-induced ethanol consumption. Thus, (-)-trans-PAT is a potential tool for treating alcoholism.

The Alcohol Deprivation Effect (ADE) is a temporary increase in ethanol consumption following a period of ethanol deprivation which can serve as a model of alcoholic relapse. Serotonergic modulators have been shown to influence voluntary consumption of ethanol and represent a potential pharmacotherapy. The ADE was studied using the “jello shot” model. The aim of this study was to attenuate the deprivation induced increase in ethanol consumption in a rat model of voluntary ethanol self-administration. (-)-trans-PAT, a novel 5-HT(2C) agonist, was shown to decrease both basal and ADE-induced ethanol consumption. Thus, (-)-trans-PAT is a potential tool for treating alcoholism.

ANALYSIS OF ANTI-INFLAMMATORY PROTEINS IN INHIBITION OF VASCULOPATHY IN LIVER ISCHEMIA/REPERFUSION INJURY MOUSE MODELS

LIVER ISCHEMIA/REPERFUSION INJURY, an innate immunity-dominated inflammatory event, remains a critical problem in clinical organ transplantation. In the specific case of the liver, IRI causes about 10% of early graft failure and can lead to a higher incidence of acute and chronic rejection. Hepatic IR damage which can occur in multiple situations such as liver transplantation, trauma, hemorrhage shock or liver surgery, is a serious clinical complication that leads to impaired liver function due to extensive hepatic cellular loss. The mechanisms responsible for hepatic injury are not well understood but some studies show that T lymphocytes are key mediators in IR-triggered liver inflammation. This research project is aimed at reducing the adverse effects of IR injury through the administration of viral protein proC. SerP and M-T7 on mice with IRI. This can ultimately lead to more successful liver transplants and decreased graft failures. Blood supply to the mice’s left/middle lobes will be blocked by clamping the portal vein and hepatic artery for 90 minutes. The clamp is then removed, initiating reperfusion and at a follow-up of 24 hours, the liver will be harvested. The protein will be administered 30 minutes before ischemia and 9 days after reperfusion by infusion into the tail vein. Immunostaining, Hematoxylin and Eosin staining other forms of staining will be used to examine the effects of the viral proteins on inflammation and hepatic cellular loss. The project is still in progress and there are only preliminary results about the group 1 mice (normal, control mice) and group 2 mice (liver ischemia mice). Results comparing their HE staining, Ki67 staining, macrophage staining, enos staining and caspase staining can be analyzed to see how liver ischemia effects the liver.

THE RELATIONSHIP BETWEEN FAT GENE EXPRESSION AND CARDIOVASCULAR DISEASE

The level of omental fat is associated with inflammation, Type II diabetes, atherosclerosis, high blood pressure, and cardiovascular disease. We hypothesize monitoring changes in omental fat gene expression is crucial for understanding progression of vascular disease and how omental fat relates to inflammation and metabolic syndrome. Inflammation of blood vessels is related to monocyte invasion into the intima. This invasion of inflammatory cells can lead to atherosclerotic plaque. M-T7, a viral anti-inflammatory protein, is a broad spectrum chemokine inhibitor that has successfully been shown to inhibit plaque growth post-balloon injury in mice. This construct and point mutants will be used in the balloon-angioplasty injury model in APOE KO mice to differentiate gene changes due to atherosclerosis, inflammation, and obesity. RNA is extracted from omental and subcutaneous fat and

that are immunocompromised are at an even greater risk of contracting this infection. Immunocompromised patients predominantly reside in the ICU units. Areas known as “touch surfaces”, such as patient bed rails, nurse calling devices and remotes, are even more prone to microbial activity. However, recent research has shown that copper has antimicrobial properties, capable of eliminating 99.9% of microbes within 2 h of exposure due to its ability to interfere with cellular physiology. Therefore, this research asks two questions: first, are nosocomial infection causing microbes more prevalent in ICU’s or non-ICU’s?, and second, how effective of an antimicrobial is copper? To address these questions, we swabbed samples from “touch surfaces” in two ICU’s and two non-ICU’s. We then added a dime, nickel, penny and quarter, all of which contain different levels of copper, to these bacteria containing media to assess its bactericidal abilities. The greatest number and variety of bacterial growth has been shown to occur in the ICU units. Nosocomial infections cost $4.5-11 billion per year to treat while Medicaid and Medicare insurance companies are opting to no longer treat these infections. This research will show that greater sanitization methods should be in place to prevent cross-transmission in the ICU where patients are more susceptible to nosocomial infections. Because controlling agent and host factors is more tedious, replacing “touch surfaces” with naturally bactericidal copper-alloys would be a far more practical method, along with proper multi-faceted hospital sanitization, in reducing nosocomial infections.

ROLE OF THE YGGs FAMILY OF PROTEINS IN PLP METABOLISM/HOMEOSTASIS

The YggS family of protein belongs to the to the Type III Pyridoxal-5-phosphate-dependent enzymes such as bacterial alanine racemase (AR), eukaryotic ornithine decarboxylases (ODC) and biosynthetic arginine decarboxylases (ADC). These AR-like proteins are typically composed of an N-terminal PLP-binding TIM-barrel domain and a C-terminal β-sandwich domain. The interface between the N- and C-terminal domains of the homodimers constitutes the active site. The YggS family of proteins (also called YBL036c-like or PROSC) is an atypical AR-like family as its members exist as monomers, lack N- or C-terminal extensions, and contain just a PLP-binding TIM-barrel domain. Proteins of this family are mostly uncharacterized. In Pseudomonas aeruginosa it is co-transcribed with the proline biosynthesis gene proC. In yeast it was shown that the YBL036c has some alanine racemase activity, in agreement with crystal structure data showing conservation of most essential residues involved in the PLP site. In order to uncover the function of members of the YggS-like proteins, we undertook a comparative genomics approach combined with genetics and phenome analysis across kingdoms. The YggS family is widely distributed among Bacteria and Eukaryotes. It is greatly underrepresented among Archaea. YggS encoding genes are found to physically cluster with proC (the gene involved in the last step of glutamate to proline conversion), genes involved in cell wall biosynthesis, aminocid biosynthesis genes (some of which encode PLP-dependent enzymes), as well as PLP-biosynthesis operons. Based on these findings we hypothesize that YggS is involved in trapping free PLP to prevent its well-known deleterious effects on cells, as well as delivering it to PLP-dependent proteins. Evidence will be presented towards this hypothesis.

IDENTIFICATION OF KEY ENZYMES IN THE PHENOLIC DEGRADATION PATHWAY OF LACTOBACILLUS JOHNSONII N6.2

Lactobacillus johnsonii N6.2 is a commensal lactic acid bacteria found in the human gut that is responsible for the release and modification of plant phenolic compounds. We have previously identified a cinnamoyl esterase in L. johnsonii N6.2 that is capable of releasing monophenolics (hydroxycinnamic acids) from plant materials often consumed in the human diet. Several monophenols (including caffeic acids) display toxic activity to many bacterial species. Therefore, the
high esterase activity displayed by L. johnsonii in cultures is at least controversial because the released monophenols can directly affect cells viability. We hypothesized that L. johnsonii imports these monophenolic compounds into the cell for further degradation. These compounds may serve as a carbon source or enter a detoxification pathway allowing the survival of L. johnsonii in the intestinal tract. L. johnsonii may benefit from the releasing of monophenolic compounds from plant cells in order to survive among other competing species. Preliminary results suggest that L. johnsonii has the ability to degrade common dietary phytochemicals (ferulic, caffeic, and p-coumaric acid). High performance liquid chromatography was used to follow degradation of monophenolic compounds. Additionally, a biochemical screening assay was conducted to determine the enzymes responsible for the degradation. The aim of this study is to identify and characterize enzymes associated with the pathway for the degradation of monophenolic compounds. This study confirmed that monophenolic compounds are imported into the bacterial cell and then consequentially undergo degradation. This process is not associated with membrane proteins but occurs via enzymatic activity within the cytosol. In addition, the activity occurs in the presence of glucose, indicating that L. johnsonii express these enzymes constitutively.

13 COMPARISON OF COMMERCIAL DISINFECTANTS AND HOUSEHOLD PRODUCTS AGAINST COMMON BACTERIAL SPECIES
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Staphylococcus aureus and Escherichia coli were exposed to both natural and commercial disinfectants. The goal was to test the effectiveness of various disinfectants on representative Gram-positive and Gram-negative bacteria. Six products were tested for their antimicrobial properties on a stainless steel surface under ambient conditions. It was proposed that if common household ingredients can be found to effectively kill bacteria on surfaces, then overall household disinfectants could be as useful as commercial disinfectants in controlling bacterial populations at home. The Use-Dilution test, an EPA approved method that tests antimicrobial efficacy, was followed as close as possible. Each bacteria-product combination was conducted in replicates of 10 with two additional controls, a positive and negative, which had carriers submerged in deionized water and bleach respectively. 48-hour bacterial cultures were dried onto stainless steel carriers and each carrier was then exposed to 10 mL of the selected disinfectant for 30 seconds. Carriers were immediately transferred to new test tubes containing letheen broth, a solution that inhibits the disinfectant but provides a growth medium for the bacteria. The solution was incubated for 48 hours and examined for growth. Determined by their turbidity, in order to pass the test, all 10 replicates required complete growth inhibition. Results revealed that commercial products inhibited growth at grader magnitudes than the natural disinfectants. Still, it was observed that Aloe Vera and vinegar had substantial inhibition on both species. Even though some natural disinfectants showed promising results, they did not pass the specified requirements. It was concluded that household products were overall not as effective as commercially available disinfectants. This work is significant because of the recent trend that is, certain cases received a relatively high score on one scale and a relatively low score on the other. We hypothesize that this difference is primarily related to variations in respondents’ spirituality. Through qualitative review of the respondents’ implicit wisdom theories, the cases of interest are compared and general trends are noted.

24 QUALITATIVE ASSESSMENT OF INCONSISTENCIES AMONG PROMINENT IMPLICIT WISDOM SCALES
P. Fine, M. Ardelt; Department of Sociology

For the past few decades, wisdom researchers have been working to determine the essential elements and characteristics of wisdom. In the current study, respondents were asked about their implicit (laymen) definitions of wisdom and also given scales that measured their relative level of wisdom. Despite general consistency, two prominent scales, ASTI (Adult Self-Transcendence Inventory) and 3D-WS (Three-Dimensional Wisdom Scale), were found to contradict one another on some cases. Our goal is to inspire prospective scientists and young, impressionable students of any age that science, and specifically, microbiology, is exciting, thought provoking, and beautiful. We believe that we need more scientists, more researchers, and more children to love science and microbes in particular. We desire that more people appreciate microbiology for not only their role in health and disease, but for the beauty of the field as a whole. We are working with the STEM + Art = STEAM movement, we are attempting to raise awareness of the intricacy and beauty of the microbial world, using the manipulation of microbes and fungi, with the goal of creating aesthetically pleasing displays of scientific art. Through this awareness, we wish to make microbiology more relatable and accessible to the common masses that have not had training in this field. Through the use of an assortment of microbiology lab tools, we have created a variety of eye-catching artistic works. These include pigments, dyes, stains, pH indicators, agars, varying strains of BSL-1 and BSL-2 organisms, and SEM and light microscopy. Some examples include the use of varying agars as a canvas for painting with microbes, and stains magnified to show the intricacy of the microbial form. In addition, the varying phenotypic responses of bacteria due to agar constituents and the resulting agar color changes due to bacterial metabolic byproducts have been manipulated to show that microbial life can be appealing to the untrained eye. Some examples of finished results include a bacterial version of the famous painting by van Gough “Starry Night”, a display of rainbow colors of agar plates, and an oriental themed Dragon painting using Proteus mirabilis on an XLD agar plate. These results will help and inspire students to understand and remember key pathogens, the agars used for identification and the scientific causes that explain why our agars change colors.

2012 Undergraduate Research Symposium Abstracts

12 RPA1 IS REQUIRED FOR NORMAL ACTIN ORGANIZATION DURING TRICHOME DEVELOPMENT IN ARABIDOPSIS
J. Roney, P. Grey, D. Oppenheimer Department of Biology

The remodeling of the actin cytoskeleton plays an essential role in many important cellular processes including intracellular transport, membrane trafficking, and cell shape control. The assembly and disassembly of the actin cytoskeleton depends on the interactions of actin monomers and actin filaments with various other proteins. Members of the actin-depolymerizing factor (ADF)/cofilin gene was assessed using quantitative real-time PCR. The growth rate of V. fischeri in liquid cultures was significantly increased under microgravity conditions with an approximate two-fold increase in cell densities. As in previously studied pathogenic bacteria, the expression of hfq in V. fischeri was also down regulated in microgravity conditions. To determine whether the loss of hfq in V. fischeri would impact the health of the host animal, juvenile E. scolopes were inoculated with V. fischeri mutants defective in hfq for up to 72 h. The phenotypes indicating colonization of the light organ was monitored, specifically the luminescence of the squid, pycnotic nuclei count and light organ cell regression. The results obtained from this experiment indicated that the microgravity environment causes changes in gene expression that in turn negatively impacts the development of the squid-vibrio symbiosia.

25 MICROART: ART AND BEAUTY FROM A MICROBIOLOGIST’S PERSPECTIVE
A. Arcea, K. Dieseldoff, I. Landrian, S. Saikaly, M. Oli
Department of Microbiology and Cell Science

Our goal is to inspire prospective scientists and young, impressionable students of any age that science is exciting, thought provoking, and beautiful. We believe that we need more scientists, more researchers, and more children to love science and microbes in particular. We desire that more people appreciate microbiology for not only their role in health and disease, but for the beauty of the field as a whole. In conjunction with the STEM + Art = STEAM movement, we are attempting to raise awareness of the intricacy and beauty of the microbial world, using the manipulation of microbes and fungi, with the goal of creating aesthetically pleasing displays of scientific art. Through this awareness, we wish to make microbiology more relatable and accessible to the common masses that have not had training in this field. Through the use of an assortment of microbiology lab tools, we have created a variety of eye-catching artistic works. These include pigments, dyes, stains, pH indicators, agars, varying strains of BSL-1 and BSL-2 organisms, and SEM and light microscopy. Some examples include the use of varying agars as a canvas for painting with microbes, and stains magnified to show the intricacy of the microbial form. In addition, the varying phenotypic responses of bacteria due to agar constituents and the resulting agar color changes due to bacterial metabolic byproducts have been manipulated to show that microbial life can be appealing to the untrained eye. Some examples of finished results include a bacterial version of the famous painting by van Gough “Starry Night”, a display of rainbow colors of agar plates, and an oriental themed Dragon painting using Proteus mirabilis on an XLD agar plate. These results will help and inspire students to understand and remember key pathogens, the agars used for identification and the scientific causes that explain why our agars change colors.
Malaria is an increasingly resistant infectious disease caused by apicomplexan protozoan parasites from the genus *Plasmodium* falciparum. It remains to be a public health issue plaguing various parts of the world, especially in underdeveloped areas in Southeast Asia and sub-Saharan Africa. Due to the lack of vaccines and a growing resistance to anti-malarials, development of novel anti-malarial drugs are of utmost importance. Rational drug development requires understanding of the biochemical pathways in the parasite’s replication cycle. The focus is on plasmepsins (*Plasmodium pepsi*ns); these proteases perform vital functions and allow the parasite to thrive. Among the ten plasmepsins, we hypothesize that PIPM9 is essential for the parasite’s survival during the asexual erythrocytic stage and is a target for the anti-malarial activity of HIV protease inhibitors (HIV PIs). These aspartic protease inhibitors prevent the normal processing of critical polypeptide substrates, killing the asexual stage of the malaria parasite (in vitro). Identifying the timing of expression and sub-cellular location of PIPM9 will help define its role and determine its potential substrates. The results will help determine if PIPM9 is a potential target for HIV PIs. Critical background information needed to test this hypothesis will be obtained by accomplishing the following specific aims: (1) Prepare, characterize, and purify monospecific polyclonal antibodies against PIPM9 from rabbit hyperimmune serum; (2) Synchronize the asexual division cycle to obtain parasites (12-hr intervals) throughout the 48-hr cycle and utilize western blot methods to detect/compare the steady-state level of PIPM9; (3) Utilize antibody preparations to localize PIPM9 in parasitized erythrocytes using immunofluorescence assay techniques; and (4) Optimize western blot to detect native PIPM9 from all proteins expressed by asexual blood-stage parasites.

**22 STUDYING THE INTERACTION BETWEEN ACTIN DEPOLYMERIZING FACTOR AND A NOVEL ADF REGULATOR**

*M. Emmanuel, P. Grey, D. Oppenheimer; Department of Biology*

The actin cytoskeleton and the actin-binding proteins are required by all eukaryotic cells to carry out key functions such as cellular motility and chemotaxis. One of the most important actin-binding proteins is actin depolymerizing factor (ADF). Earlier work in our lab led to the identification of a novel regulator of ADF in plants, called IRREGULAR TRICHOME BRANCH 3 (ITB3). In this study we will quantify the interactions between ITB3 and ADF, an ITB3-like family member, and ADF. This will allow us to confirm ITB304’s effect as a regulator of ADF and characterize this new pathway in actin dynamics.

**23 MICROGRAVITY-INDUCED CHANGES IN THE EXPRESSION OF THE GLOBAL REGULATOR HFQ IN THE MUTUALISTIC BACTERIUM VIBRIO FISCHERI**

*K. Grant, C. Khodadad, J. S. Foster; Department of Microbiology and Cell Science*

All animals form associations with beneficial microbes and it is becoming increasingly apparent that the co-existence between animals and their microbial consortia is essential for health and normal development. In space flight, however, it is unclear how these associations with mutualistic microbes are impacted. In this study we examined the impact of simulated microgravity on the expression of hfq in the mutualistic bacterium *Vibrio fischeri* to determine whether there are changes in virulence in this non-pathogenic organism. To simulate microgravity conditions we used high-aspect ratio rotating wall vessel (HARV) bioreactors and maintained *V. fischeri* cultures for up to 48 h. Parallel gravity controls were conducted for all experiments. Cultures were grown in seawater tryptone broth and samples were collected every 2 h and preserved in RNAlater® until extraction. Expression of the hfq protein family regulate actin dynamics by severing actin filaments and increasing disassembly. Because ADF is the main protein involved in actin depolymerization, understanding ADF regulation is important for understanding the regulation of actin dynamics. Recently, a novel Regulator of Plant ADF (RPA1) has been shown to bind to and inhibit plant ADF function. Mutations in the RPA1 gene encoding a member of the RPA protein family of *Arabidopsis thaliana* show defects in epidermal hair (trichome) shape, suggesting disruption of the actin cytoskeleton. Here we show the impact of the absence of RPA1 on actin organization in rpa1-1 and rpa1-2 mutants using fluorescent phalloidin and confocal microscopy. At stage 5 of trichome development, we observe highly irregular actin organization. We observe many actin rings near the nucleus. In addition, we found that rpa1-1 and rpa1-2 mutants have actin filaments with little overall alignment compared to wild type. The results from this study demonstrate that RPA1 is necessary for normal actin organization in plant trichomes.

**15 GROWTH OF SALMONELLA ENTERICA SEROVAR TYPHIMURIUM IN CONVENTIONAL AND ORGANIC TOMATOES**

*C. Reist, K. Weiss, J. Yarbrough, M. Oli; Department of Microbiology and Cell Science*

Tomatoes (*Lycopersicum esculentum*) are known for is their strong antioxidant properties, attributed to lycopene and polyphenols. However, tomatoes are also known for causing food poisoning due to *Salmonella* contamination. Scientific evidence indicates that organic tomatoes have more antioxidants compared to inorganic produce. In this study we compared growth of *Salmonella enterica* serovar Typhimurium in organic and inorganic tomatoes. Tomatoes were inoculated with *Salmonella* and a rpoS mutant. The rpoS mutant shows increased susceptibility to oxidative stress and is thought to be more affected by the antioxidants in the tomatoes. Bacterial growth was determined over a period of time (72 h and 144 h) on XLD agar. Preliminary data suggest that organic tomatoes show reduced growth of the food poisoning pathogen. Further studies are needed to determine whether increased antioxidant content in organic tomatoes contributes to reduced risk of food poisoning of organic produce.

**16 PROCESSING NON-CANONICAL DNA STRUCTURES BY TRANSCRIPTION**

*S. Nguyen, S. Tornaletti; Department of Anatomy and Cell Biology*

Our research investigates the effects of the transcription-coupled DNA repair process on a genomic site capable of forming non-canonical DNA structures. DNA sequences that can form structures that deviate from the canonical, helical duplex B DNA structure often correspond to genomic regions that are highly susceptible to genetic instability, an early contributing factor in human disease and cancer development. DNA transcription through these regions plays a central role in the promotion of these structures’ formation and in the mutagenesis associated with these sites. We hypothesize that because of the unusual structural features of non-canonical DNA, these structures may arrest transcription. To test this model, the effects of transcription will be investigated in biologically relevant sequences that form quadruplex DNA structures identified in the c-myc gene. G-quadruplex (G4) DNA forms in DNA sequences with guanine (G) repeats and consists of planar arrays of four hydrogen-bonded guanines called G quartets. This structure is stabilized by physiological concentrations of KCl and PEG. The G4 forming sequence from the c-myc promoter will be cloned downstream of the T7 or the T5 promoter. We will use restriction enzymes to cut the DNA plasmid. Next, we will transcribe the plasmid using a single subunit enzyme, T7 RNA polymerase (T7 RNAP), as a model to measure the extent of the transcription arrest. Transcription was arrested in the exact location that we expected the G4 structure to be present, confirming the structure’s presence and ability to arrest transcription.
FORMATION OF RENAL CRYSTALS AND THE SUBSEQUENT ACTIVATION OF THE INFLAMMATORY RESPONSE AS A PRECURSOR TO RENAL FAILURE IN RAT MODEL

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Over the last three decades many studies have been done on crystal formations and kidney failure. However most of these studies were therapeutically based, focusing on drug formations and safe organ transplant. Furthermore while we now know the possible substances and mechanism of how these crystals form, the immediate biochemical consequences in the body that are directly responsible for kidney failure is still unclear. In this experiment we attempt to understand the role of an activated immune response mediated by the presence of crystals in support of our hypothesis that the real damage is actually caused by the NADPH-oxidases reactions. To set up our experiment we first divided syngeneic rats into two groups, a control group and the experiment group with an oxalate inducing agent hydrox-L-proline (HLP) added to 5% ethylene glycol (EG) in water at 0.75-1.25%. In addition the experimental rats were treated with one of the three anti-inflammatory agents with different concentrations. We also divided each group with 5 different time frames after which renal tissues were collected for microscopic and histological evaluations (day 7, 14, 28, 42, 120). Immunoassay done on day 21 and 28 with four different Antibodies were recently conducted. These Antibody molecules include FOX2, FOX4, P22 and MCP1. We found that all four of these molecules bind to the tissues; indicating a very active immune response in the tissue. The high level of immune-activity also suggests the presence of NADPH-oxidases reactions (that are attempting to remove the crystals.) Because the crystals are constantly growing more attempts are made to remove it resulting in hyperactive immune-response. Unfortunately in the process the constant immune response specifically NADPH-oxidases on the tissue results in causing more damage and faster degradation of cells than the crystals themselves.

ACTIVITY OF TSTR TRANSCRIPTIONAL REGULATOR IN LACTOBACILLUS BREVIS IS MEDIATED BY INTERACTION WITH IRON

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The ability of small molecules to modulate the activity of transcriptional regulators is an emerging area of study. In this regard, TstR from Lactobacillus brevis is a transcriptional regulator of the MarR family that has been shown to interact with small molecules like iron. Structurally, TstR displays an unusual C-terminal domain with the presence of a non-conserved cysteine residue. In the genomic context of TstR immediately downstream is coded Tst, which exhibits a putative thiolase: cytidine sulfurtransferase or rhodanese domain. The goal of this study was to elucidate the mechanism through which TstR regulates the expression of TstR and downstream genes in the TstR-Tst operon. By in silico prediction we determined that the TstR-Tst operon structure consists of a promoter in the 5' region of TstR and a rho-independent terminator in the 3' region of Tst. Through electrophoretic mobility shift assays (EMSA) and DNase I footprint experiments we confirmed that TstR binds with high affinity within its promoter region (PTstR). It was determined that iron influenced TstR binding to PTstR. EMSA assays showed that TstR interaction with PTstR is impaired in presence of increasing concentrations of Fe(II), up to 50μM. To characterize the regulatory effect of the TstR:PTstR interaction, β-galactosidase assays were used to measure the relative transcriptional activity of TstR in the presence of differing concentrations of iron and iron-chelating agents. Different constructions containing lacZ as a reporter gene were cloned into pDG1663 plasmid and transformed for ectopic insertion in Bacillus subtilis. Preliminary data shows that LacZ activity is induced by two-fold in the presence of iron. Collective data from EMSA experiments and β-galactosidase assays suggest that TstR acts as a repressor on the TstR-Tst operon, consistent with other MarR homologs.

RNA-Seq is a tool used for assessing gene expression based on read counts from high throughput sequencing. Many analysis methods to detect differential expression thus far have focused on using the Poisson distribution or the negative binomial distribution for analysis of differential expression. Looking at both the underlying data and measurement on which the analysis is performed, we propose that RNA-Seq data can be considered continuous. The underlying libraries are made from a solution of mRNA quantified by concentration. This solution is sampled and sequencing technology is used to estimate the number of molecules in the sample for a particular gene. Normalization techniques, which result in non-integer values, are often applied to RNA-Seq data in order to account for systematic effects on the total number of counts, for example the length of the exon/transcript and the total number of reads mapped to the reference per sample. Examination of four different experiments and using a general linear model with a normal distribution reveal the residuals do conform to underlying assumptions when data are complete. When some observations are missing the residual assumptions are often violated. The effect from these missing observations on the model and significance tests can be best avoided through an imputation technique.

CLONING OF THE SHORT EFFICIENT PROMOTER FOR SPECIFIC TRANSDUCTION OF MONOCYTE-DERIVED DENDRITIC CELL BY ADENO-ASSOCIATED VIRUS VECTORS

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Despite aggressive conventional treatment regimens, survival rates for patients with different types of cancer remain relatively low. Dendritic cell (DC) based immunotherapy represents an attractive alternative for currently used anti-cancer treatments. Adeno-associated virus vectors (AAV) pose several advantages compared to these methods for DC loading in terms of efficiency and longevity of antigen presentation. The use of specific promoters represents an important approach for immunization to limit gene expression to target cells and address safety concerns associated with viral vector-based vaccines. The packaging capacity of these vectors (approximately 2.4 kb) is significantly small. In the present studies we analyzed the promoter that drives CD11c expression, a common co-stimulatory molecule among different subsets of DC, and identified a functionally sufficient region, which can fit into the AAV expressing cassette. Five different regions with lengths around 700bp were chosen from the full length CD11c promoter: CD11c1-3505-2802 - CD11c1-3505-2802 and CD11c1-2131-1429. Fragments were amplified from genomic human DNA and then cloned to drive EGFP expression. Each construction was packaged into adeno-associated virus vector serotype 6 (AAV6). Each construct was evaluated for ability to express EGFP in monocyte-derived dendritic cells (mDC). We showed that two short promoter parts CD11c1-3505-2802 and CD11c1-2131-1429 can drive EGFP expression with comparable efficiency with commonly used CD promoter. Next we used freely available software chip-mapper.org to identify possible putative binding sites for dendritic cell specific transcriptional factors which are the likely cause of functional activity of a particular part of CD11c promoter. Analysis revealed several binding motifs on CD11c1-3505-2802 and CD11c1-2131-1429 for transcriptional factors associated with DC development and maturation such as PU1, Ikaros, Ap1, Sp1 and NF-kB. Taken together, sufficient part of the human CD11c promoter allows for specific targeting of mDC and represents a promising tool for immunotherapy.